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INFLUENCE OF NUTRITION AND DIFFERENT PHYSICAL PARAMETERS ON GROWTH AND SPORULATION OF *METARHIZIUM ANISOPLIAE*

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Abstract: Present investigation was carried out to find out the effect of different parameters on growth and sporulation of *Metarhizium anisopliae* . Amongst nine media tested SDA+Y media was found most suitable for growth and sporulation of *M.anisopliae*. The temperature 25^0C followed by room temperature was found most suitable. During the incubation of mass culture relative humidity of 85% favours the growth and sporulation. The best pH 5.5 was found best and recorded maximum mycelial growth with abundant sporulation.

Introduction

A biological control plays an important role in integrated pest management and non pesticide pest management programme (Saxena and Ahmad, 1997). Integrated pest management is essential to minimize the use of synthetic pesticides, which must be safer to human beings and natural enemies so as to balance the ecosystem and conserve the biodiversity. The numbers of microorganisms like fungi, bacteria, protozoa, nematodes are known to parasitic on insect pest. Under natural condition, fungi are frequent and often important natural mortality factors in insect population. Over 700 species of fungi have been reported to be pathogenic on insects. Among several existing entomopathogenic fungi. *Metarhizium anisopliae* is getting more attention in IPM programme. The success of biocontrol technology depends on its mass production, and physical parameters and their effective utilization in fields.

Material and Methods

The pure culture of *M.anisopliae* was obtained from Department of Plant Pathology, College of Agriculture, Nagpur, and it was purified by single spore isolation. A loop full suspension of test fungus made in sterile distilled water mixed with 15 ml of 20 % melted SDA+Y in test tubes then it was poured in petriplates, after solidification the plates were incubated at 27 ± 1^{OC} for 96 to 120 hrs. the isolated colonies were well marked and lifted

aseptically on Sabourauds dextrose agar slants. After achieving desired growth at room temperature it is further multiply for studies.

Effect of Temperature

Effect of temperature was studied by incubation the inoculated plates at 15, 20, 25, 30, 35 and 40 ^{OC} in incubator and room temperature. The comparable uniform colony diameter was measured after seven days. The experiment was conducted in four replications.

Effect of humidity

The inoculated plates were exposed to 75, 80, 85, 90, 95 and 100RH levels in desiccators. The humidity was maintained by using concentrated sulphuric acid + water as suggested by Soloman (1951).

Effect of light

The inoculated plates were exposed to continuous light provided by two 4OW white fluorescent tubes arranged 41 cm above plates, alternate cycle of 12 hr light and darkness, complete darkness provided by wrapping black paper from inner and outer side of the bell jar. Radial growth was measured after seven days.

Effect of pH

Different pH range 5.5, 6, 6.5, 77.5, 8 and 8.5 were tested for the growth and sporulation of *M. anisopliae* by adjusting the pH with NaoH and HCL. The pH of media was adjusted from 5.5 to 8.5 autoclaved and poured in plates allow to solidify. Each plate was inoculated with 5 mm disc of test fungus. The radial mycelia growth was measured at seven days after incubation.

Testing different media

The cultural studies were carried out using the various solid and liquid media. Sabourauds dextrose, Emmersony yeast phosphate soluble starch media, Potato dextrose, Cotton cake extract, luria were tested.

Results and Discussion

Nine different liquid and solid medias were tested to determine fevourable media to promote the growth and sporulation of test fungus (Table 1). Amongst them M2 was found best (0.566 and 33.21 mm) for both sporulation and growth respectively. The efficacy of other media were M9 (0.385), M4 (0.345g), M3 (0.0.303 g), M8 (0.273 g), M7 (.245g), M6 (0.260)and M5(0226). With regards to sporulation at 15 days interval PDA, SD+Y, EYPSS, jiggery yeast extract supported maximum sporulation. In case of solid media M2 (0.33.21

mm) was found best followed by cotton cake extract, EYPSS, Luria agar. Least growth was recorded on PDA. Yewale (2001) and Burgoni (2005) reported the same results.

Temperature is an environmental factor having significant effect on growth and sporulation of fungus. The most favorable temperature 25c giving maximum colony diameter (25.00). Minimum growth was recorded at 350c (6.5) as well as 15oc (10.86 mm). However at 400c no growth was observed (Table 2). Yewale (2001) reported that optimum temperature required for growth and sporulation was 25 0^{c}.

Table 1: Effect of different liquid and solid media on growth and sporulation on *M.anisopliae*

Sr	Name of media	Medium	Liquid media		Solid media	
			Dry mycelia weight after 10 days (g)	Sporulation after 15 days	Colony diameter after 7 days (mm)	Sporulation after 15 days
1	Emerson Yeast Phosphate Soluble starch medium	M 1	0.450	++++	30.00	++++
2	Saboraud's agar + yeast medium	M 2	0.566	++++	33.21	++++
3	Potato dextrose agar	M 3	0.303	++++	11.75	+
4	Cotton cake extract medium	M 4	0.343	+++	30.64	+++

5	Cotton leaf extract medium	M 5	0.226	-	28.52	+
6	Jowar meal extract medium	M 6	0.260	+	22.00	+++
7	Luria agar nedium	M 7	0.245	+++	29.25	++++
8	Cotton seed extract medium	M 8	0.273	++	27.00	+
9	Jaggery yeast extract medium	M 9	0.385	++++	26.87	+
F test			Sig		Sig	
CD (p= .05)			0.033		1.70	

Table 2: Effect of different levels of temperature on growth and sporulation of *Metarhizium anisopliae*.

Treatment	**Temperature (oc)**	**Colony diameter after 7 days (mm)***	**Sporulation after 15 days**
T 1	15 ^{0}C	10.86	+
T2	20 ^{0}C	21.75	+++
T3	25 ^{0}C	25.00	++++
T4	30 ^{0}C	17.48	+++
T5	35 ^{0}C	6.54	++
T6	40 ^{0}C	00.00	-
T7	Ambient temp	22.23	+++

F test		Sig	
CD (p= 0.05)		1.89	

* Average of four replications

With regards to humidity 85 and above upto 100% has given higher colony diameter with range to 36.80 to 35.40 mm with abundant sporulation. There was no significant difference observed at 85, 90, 95 % (Table 3). Similar observations were recorded by jairamaiah and Veeresh (1982).

Table 3: Effect of different levels of humidity on growth and sporulation of *M.anisopliae*

Treatment	Relative humidity levels %	Colony diameter after 7 days (mm)*	Sporulation after 15 days
T 1	75	32.60	+++
T2	80	34.20	+++
T3	85	36.80	++++
T4	90	37.00	++++
T5	95	37.80	++++
T6	100	35.40	++++
F test		Sig	
CD (p= 0.05)		2.07	

Average of five replications

Regarding effect of light on growth and sporulation of test fungus (Table 4), alternate light and darkness for 12 hrs showed significantly superior linear growth (35.00 mm) with abundant sporulation over remaining two sources.

Table 4: Effect of different light condition on growth and sporulation of *M.anisopliae*

Treatment	Particulars of source of light	Colony diameter after 7 days (mm)*	Sporulation after 15 days
T 1	Alternate cycle of 12 hr light and 12 hr darkness	35.00	++++
T2	Continuous darkness	30.85	+
T3	Continuous light	32.57	+
F test		Sig	
CD (p= 0.05)		1.76	

Effect of pH (Table 5) indicated that highest radial mycelia growth was recorded at pH 5.5 (35.66 mm) followed by 6.0 (33.00mm), 6.5 (33.66 mm). The lowest mycelia growth was recorded at pH8.0 (27.3 mm).

Table 5: Effect of different pH on growth and sporulation of *M.anisopliae*

Treatment	pH	Colony diameter after 7 days (mm)*	Sporulation after 15 days
T 1	5.5	35.66	++++
T2	6.0	33.00	++++
T3	6.5	33.66	++++
T4	7.0	32.00	+
T5	7.5	32.66	+
T6	8.0	27.33	+
T7	8.5	28.66	+
F test		Sig	
CD (p= 0.05)		2.89	

Average of three replications.

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