Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5156

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (2):** 821-825 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Evaluation of Different Types of Media for Better Growth of the *Botryiodiplodia theobromae* Pat.

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Received: 9.07.2017 | Revised: 22.08.2017 | Accepted: 1.09.2017

ABSTRACT

Rose (Local/ Deshi) is one of the important flower crops and grown more or less in North Gujarat. Among different diseases, die- back of rose caused by Botryodiplodia theobromae is one of the most dreaded diseases throughout the North Gujarat. Considering the seriousness of the problem, the present investigations were carried out to generate more information for developing suitable control measures. Out of ten solid and liquid media tried, carrot root agar, Richard's medium and potato dextrose were proved best for the mycelial growth of the fungus. In liquid media Richard's medium, rose twig agar, Czapek's medium potato dextrose agar were produced maximum dry mycelial weight and maximum sporulation produced by Richard's agar, oat meal agar and Czapek's medium.

Key words: *Rose, Medium, Oat meal, Sporulation*

INTRODUCTION

Rose (*Rosa* spp., Family: Rosaceae) is one of the nature's beautiful creations and is universally acclaimed as "Queen of flowers". No other flower is a better symbol of love, adoration, innocence and other virtues than the rose and not in our time only but so it has been for thousands of years. It is certainly the best known and most popular of all the garden flowers throughout the world and has been growing on this earth for many million years before man himself appeared[4, 5]. Rose has become the part and parcel of life, being connected with all phases of life right from birth to death. A large quantity of rose flowers is used for decorative purpose. Besides it is used for making essence, rose water for flavoring sweets and other food articles as well as sprinkle for welcoming guests on festive occasions. Hips of some rose species are rich in vitamin C while its petals are used for preparing gulkand and pankhuri- two food articals of delicacy[3]. In india, rose is cultivated in all regions and almost all big cities such as Mumbai, Jammu, Dehradun, Nagpur, Simla, Srinagar, Indore, Banglore, Kasuali, Mysure, Udaipur, Delhi, Chandigarh, Lucknow, Poona, etc[6]. and it was occupied approximately 20 per cent of the total area under ornamental plants[11].

Cite this article: Kumar, H., Patel, D.S. and Ola, C.M., Evaluation of Different Types of Media for Better Growth of the *Botryiodiplodia theobromae* Pat., *Int. J. Pure App. Biosci.* **6(2):** 821-825 (2018). doi: http://dx.doi.org/10.18782/2320-7051.5156

Table 1: Suitable media for *B. theobromae* reported by various workers

Sr. No.	Isolate	Media	Remarks	Author (s)
1.	Rose	PDA, Oat meal agar medium and rose leaf extracts PDA, Richard's medium and Czapek's medium	Found to best for mycelial growth , best for sporulation	Mohod[7]
2.	Rose	Carrot root agar, chilli twig agar, Richard's medium and Oat meal agar	Found to best for mycelial growth, Best for sporulation	Dambhla[2]
3.	Mango	Richard's medium	Found to the gest for growth and sporulation	Shelar *et al*[10],
4.	Sapota	PDA, Richard's medium and Czapek's medium	Best for mycelial growth	Patel[8]
5.	Papaya	Papaya dextrose agar	Maximum growth	Siradhana[12]
6.	Citrus	Czapek's medium	Showed best growth	Bhatnagar[1]

MATERIAL AND METHODS

With a view to find out the best media for the growth and sporulation of pathogen, following media in solid and liquid state were used.

1. Carrot root medium
2. Oat meal medium
3. Potato dextrose agar
4. Richard's medium
5. Brown's medium
6. Czapek's (Dox) medium
7. Glucose asparagin medium
8. Asthana and Hawker's medium
9. Mango twig extract medium
10. Rose twig extract medium

Twenty ml of each sterilized agar based medium was poured in each of 90 mm diameter sterilized Petri plates and allowed to solidify. The Petri plates were inoculated by cutting and placing 5 mm culture block, aseptically in the centre from the 15 days old pure culture of pathogen. Four replications of each medium were kept and then Petri plates were incubated at room temperature ($27^o \pm 2^oC$). In all the cases of pH was adjust to 6.5 before adding agar. Observation for the linear growth, pycnidial production and colony characters were recorded periodically

Linear growth and colony characters were recorded regularly at an interval of 24 hours. Four counting pycnidia, randomly five uniform agar bits of 1 cm^2 size were taken with help of scalpel on slides from various area of the culture plates and slide was put on colony counter with the help of pointer, number of pycnidia present in each were counted and recorded. All the solid media mentioned earlier were used as liquid media having the same ingredient extract agar. This media was dispensed in 150 ml sterilized conical flasks keeping 50 ml medium per flask. The pH of each media were adjusted to 6.5 with the help of Backman's pH meter. Then all the flasks were plugged with non-absorbent cotton and autoclaved at 1.2kg cm^{-2} pressure for 20 minutes. These flasks were then inoculated by placing 5 mm diameter culture block, cut aseptically from 15 days old culture of *B. theobromae*. Each medium was reported four times then all the flasks were incubated at room temperature. Observation for dry mycelial weight, final pH and sporulation were recorded after 15 days of inoculation. Mycelial mats were harvested by filtering in on previously weighted Whatman's filter paper No. 42, giving sufficient washing with warm distilled water. The filter papers with mycelial mats were dried in oven at 80°C for 48 hours and dry weight of mycelium was recorded. Final pH in each flask were recorded.

For counting the spores, extra 50ml sterilized water was added in to the flasks and homogenized. A drop from all these was placed on the slide and number of spores per low power microscopic field (100 X) were counted, replicating 4 times in each drops.

RESULTS AND DISCUSSION

Ten different media including semi-synthetic, synthetic and natural (non-synthetic) in solid and liquid state were tested for their suitability to the growth and sporulation of the fungus *B.*

theobromae. Colony characters, linear growth and pycnidial formation were recorded from agar medium whereas, dry mycelial weight, number of spores and effect on final pH were recorded in liquid medium.

It is evident from the results presented that among all the solid media tested; significantly maximum mycelial growth (90mm) was recorded on carrot root agar medium, which was at par with potato dextrose agar (86.50 mm). The next best in order of merit were Richard's agar (85.83 mm) and mango twig agar (79.42 mm) medium, which were at par with each other and significantly superior than rest. Pycnidial formation was significantly higher in case of oat meal agar (115 pycnidia/sq. cm) as compared to rest. The next best was Richard's agar (106 pycnidia/sq. cm) followed by PDA (86 pycnidia/sq. cm), which also yielded significantly more pycnidia as compared to the remaining.

In the liquid media significantly maximum dry mycelial weight was obtained in Richard's medium (1224 mg), which was significantly superior over rest of the media tested. The next best was rose twig medium (1026 mg) followed by Czapek's medium (975 mg) potato dextrose (973 mg) and oat meal (785 mg) from which significantly higher dry weight were obtained then the rest of media tested.

Considering sporulation in liquid media, maximum spores per optical field (100 X micro field) were observed in Richard's medium (55 spores/ field). The next best was oat meal (46 spores/field) followed by Czapek's medium (35 spores/field), mango twig medium (28 spores/field) and potato dextrose medium (25 spores/field). Least sporulation was observed in Brown's and Glucose asparagine media. In all the liquid media the final pH was neutral to the alkaline.

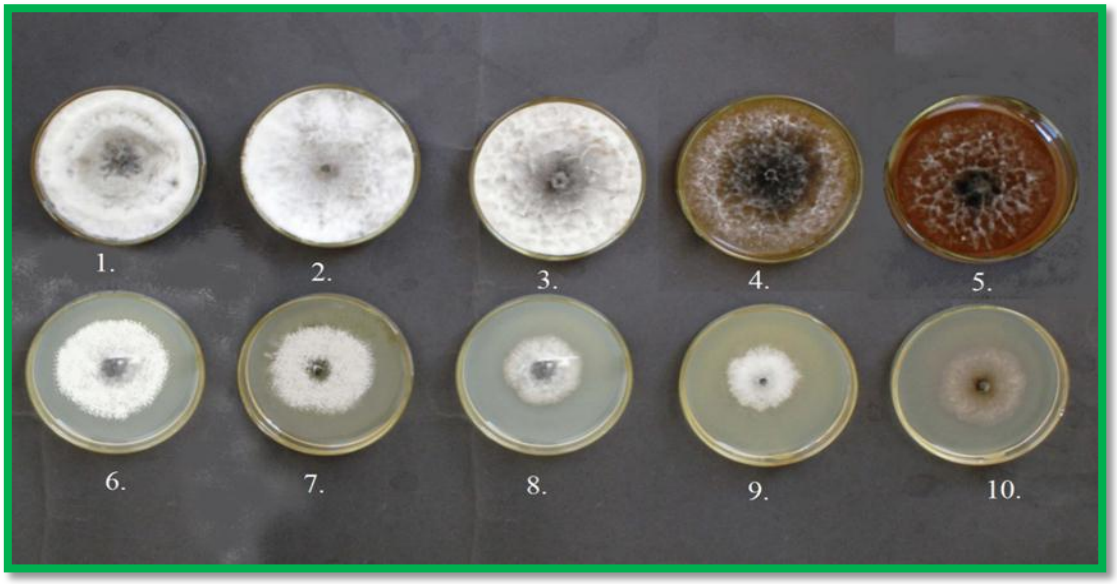
Table 2: Effect of liquid media on dry mycelial weight, sporulation and pH change

Sr. No.	Name of the media	Dry mycelial weight (mg)	No. of spores/ optical field	Final pH
1.	Carrot root medium	520	15	7.5
2.	Rose twig medium	1026	32	7.0
3.	Mango twig medium	564	28	7.5
4.	Richard's medium	1224	55	7.5
5.	Potato dextrose medium	973	25	7.0
6.	Czapek's medium	975	35	8.0
7.	Oat meal medium	785	46	7.5
8.	Brown's medium	134	10	7.0
9.	Asthana and Hawker medium	510	14	7.5
10.	Glucose asparagine media	426	10	8.0
	S. Em. ±	3.35	0.54	
	C. D. AT 5%	9.96	1.63	
	C. V. (%)	1.02	3.52	

Table 3: Effect of different solid media on linear growth, pycnidial production and colony characters of *B. theobromae*

Sr. No.	Name of the media	Linear growth (mm)	No. of pycnidia per sq. cm.	Colony characters
1.	Carrot root medium	90.00	31	Initial pale yellowish white fluffy growth, touch to the lid of the dish at periphery. Later turned olivacious. Pycnidia visually observed
2.	Rose twig medium	86.00	33	Regular margin, Initial cottony fluffy growth with blakish centres and later turned blackish. Pycnidia not visually observed
3.	Mango twig medium	83.33	52	Whitish depressed flate growth and later on turned to dirty white to olivacious Initial centre remained transparent and afterward blackish. Pycnidia visually observed
4.	Richard's medium	90.00	106	Cottony fluffy effused mycelial growth, later on much growth produced which touched the upper lid of the dish, oblivious to black in colour, under surface was quite dark, pycnidia small sized and not observed visually.
5.	Potato dextrose agar	90.00	86	Initial flate, cottony white in colour, which turned into oblivious. Pycnidia were observed visually from both the surface, not well disturbed, but more in the centre and less towards periphery.
6.	Czapek's medium	79.00	66	Fluffy, white to dirty white colony initially: later on turned olivacious in colour. Pycnidia were small and difficult to count.
7.	Oat meal medium	66.67	115	Regular margin, Initial cottony fluffy growth with blackish centres and later turned blackish. Pycnidia not visually observed
8.	Brown's medium	42.67	10	Flate, hyaline mycelial growth which changed to dirty white. Pycnidia were scattered, visible and countable.
9.	Asthana and Hawker medium	40.67	55	Blackish with white fluffy growth , 2 zonal, substrate growth, semi- fluffy
10.	Glucose asparagine media	37.67	62	Cottony white with semi-fluffy growth, substrate with big white margin.
S. Em. ±		0.44	0.65	
C. D. AT 5%		1.31	1.92	
C. V. (%)		1.08	1.82	

Plate: Effect of different solid and liquid media on linear growth and colony character of *B. theobromae*



Sr. No.	Name of the media	Sr. No.	Name of the media
1.	Carrot root agar	6.	Czapek's agar
2.	Richard's agar	7.	Oat meal agar
3.	Potato dextrose agar	8.	Brown's agar
4.	Rose twig agar	9.	Asthana and Hawker media
5.	Mango twig agar	10.	Glucose Asparagine media

DISCUSSION

The studies on testing of various natural (non-synthetic), synthetic and semi-synthetic and liquid media for their suitability to the growth and sporulation of *B. theobromae* suggested that among the solid media Carrot root agar, Richard's media, potato dextrose agar and rose twig agar were found best for vegetative growth followed by Mango twig agar and Czapek's (Dox) Agar (CzDA), and it is new

information. Considering the sporulation in natural media, the maximum sporulation was observed in oat meal agar followed by mango twig agar and rose twig agar both in solid and liquid state. Sabalpara[9] reported that oat meal media proved best for growth and sporulation of *B. theobromae*. Our findings are in line with the above worker.

The semi-liquid medium PDA also proved superior for the growth and sporulation of *B. theobromae* in solid and liquid state. This is in agreements with the findings of earlier workers[8,9].

Among the synthetic media tested, Richard's media was proved best for the growth and sporulation of *B. theobromae* followed by Czapek's (Dox) Agar (CzDA) both in solid and liquid state. Siradhana[12] found that maximum dry mycelial weight was observed in liquid Richard's medium. Bhatnagar[1] reported that Czapek's medium showed best growth of *B. theobromae*. Sabalpara[9], Patel[8] and Shelar *et al.*[10], reported that Richard's medium was proved best for growth and sporulation of *B. theobromae*. Our findings are in line with the earlier workers.

Brown's media, Asthana and Hawker media and glucose asperagin media found to be poor for the growth and sporulation of *B. theobromae* both in solid and liquid state. This is in conformity with Sabalpara[9].

SUMMARY AND CONCLUSION

Out of ten solid and liquid media tried, Carrot root agar, Richards medium and Potato dextrose agar were proved best for the mycelial growth of the fungus. While oat meal agar, Richard's medium and potato dextrose agar proved best for sporulation. In liquid media Richard's medium, rose twig agar and Czapek's medium were produced maximum dry mycelial weight, while maximum sporulation produced by richards's agar, oat meal medium and Czapek's medium.

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