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STANDARDIZATION OF SPECIFIC MEDIA FOR *PHYTOPHTHORA INFESTANS*

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ABSTRACT
The *Phytophthora infestans* is one of the major disease causing organism. This is also known as "plant destroyer". It severely attacks to the solanaceae family, like late blight in potato and tomato. For the morphological and molecular studies growing of this pathogen in *in vitro* is very necessary. As per reports there are five different artificial media which are used for growing the *Phytophthora infestans*. Many reports have stated Rye A agar (Himedia), Rye B agar (Himedia), V8 juice agar (Himedia) and Potato dextrose agar were used for the growth and to determine the characteristics of the pathogen. But these media were shown very less response for the fungal growth. Finally a new composition Carrot Sucrose Agar (CSA) Media was identified for the growth of *Phytophthora infestans*.

KEY WORDS: *Phytophthora infestans*, culture media, late blight, Carrot Sucrose Agar (CSA) medium, plant destroyer.

INTRODUCTION

Late blight is an important disease of potatoes and tomatoes worldwide which was caused by oomycete *Phytophthora infestans* (Jaimasit., P and Prakob, W., 2011). It was first appeared in 1840s, few decades ago late blight has become devastating disease worldwide (Goodwin *et al*., 1994a; Goodwin *et al*., 1994b). The symptoms of the pathogen will start by producing water soaked lesions with chlorotic borders initially these are very small in shape but in suitable humid condition it expands very rapidly, and entire plant will attacked by the pathogen within few days and automatically the heavy loss in the tubers yield (Flier, *et al.,* 2001). And this automatically leads to the total yield loss throughout the world. Estimations states that about € 12 billion of crop per annum were losses due to *Phytophthora infestans* and in developing countries it is around € 10 billion per annum (Haverkort *et al*., 2009). As per survey for the control of late blight of potato the United States revealed that use of fungicides alone costs $77.1 million at an average cost of around $507 per ha and the non-fungicide control practices are not included (Guenthner *et al*., 2001). The region wise crop loss statistics of late blight is announced by CIP 1997, the regions like Sub-Saharan Africa (44% crop losses), Latin America (36%), Caribbean (36%), South-East Asia (35%), South-West Asia (19%) and Middle East and North Africa (9%) (CIP., 1997). In India loss of crop yield by the late blight is 10 to 75% (Peerzada *et al.,* 2010). Some existing reports are available for the evaluation of culture media for *Phytophthora colocasiae* which is causal agent for taro leaf blight (Tsopmbeng *et al*., 2012). For the molecular level studies growing of this fungus is very necessary (Arora *et al.,* 2014).

MATERIALS & METHODS

Preparation of media

1. Wash 220gms of fresh red carrot and cut into small pieces.
2. Autoclave cut carrot pieces in 500ml of double distilled water.
3. Wear the gloves and blend the warm carrot.
4. Squeeze the homogenate mixture with 3-4 layered muslin cloth.
5. Volume of the homogenate mixture were make up to 1liter with double distilled water.
6. Add 20gms of sucrose and 9gms of agar (Bacteriological-Himedia).
7. Sterilize by autoclaving at 15 lbs pressure (121 °**C**) for 20 minutes.
8. When the media reaches to the 45-50°C pour into the petri plates.

Isolation and purification of pathogen

The samples of late blight infected plant leaves were obtained from National Horticulture Mission Block College of Agriculture Hassan. These Infected potato leaves were collected and transferred on to sterile petri plate and kept at 22°C for the initiation of mycelial growth and then transferred to freshly cut potato pieces in sterile condition and incubated at 22°C after 5-6 days mycelium and spores that grew out. This potato cut pieces inoculated to the newly composed Carrot Sucrose Agar medium and Rye Agar medium. Incubated at 22°C then sub cultured and purified on another Carrot Sucrose Agar plate and Rye Agar plate. (Table No.1).

Microscopic observation of sporangia:

1. Take a clean slide and put a drop of sterile water on the surface of the glass slide.
2. 15-20 day's old culture was taken with the sterile needle.

3. And transferred to the glass slide containing drop of sterile water.
4. Place the cover slip over the water drop and observed under light microscope at magnification 40 X.
5. Based on colony morphology and characteristics of sporangium and oospores the pathogenic fungi were identified.

TABLE 1: Composition of carrot sucrose agar medium and rye agar medium

Carrot Sucrose Agar media		Rye Agar Media	
Ingredients	g/L	Ingredients	g/L
Carrot	220	Rye seeds	60
Sucrose	20	Sucrose	20
Agar	9	Agar	9

RESULT

After the inoculation of the *Phytophthora infestans* to the freshly prepared carrot sucrose agar and Rye Agar media, on the surface of the media whitish mycelia were observed after 5-6 days. The fungus grew on both the media *viz* Rye Agar and Carrot Sucrose Agar medium (Fig 1). Growth of the pathogen on both media was almost similar. For further identification microscopic examination was carried out at 40 X magnification of the culture on both the media showed that sporangia are hyaline (clear) and lemon-shaped (Fig 2).

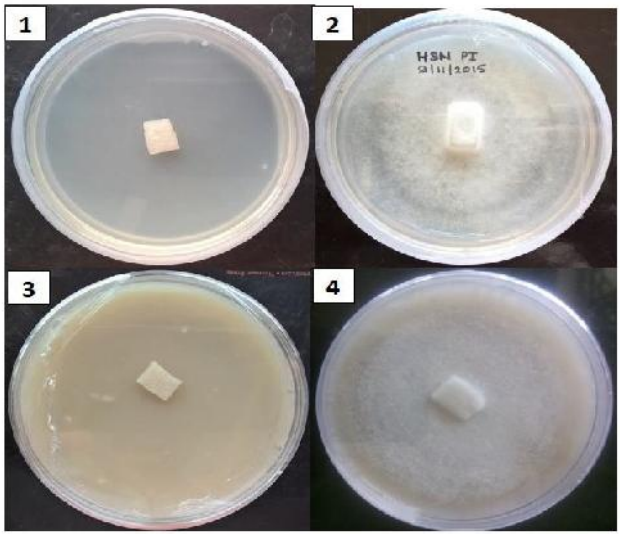

FIGURE 1: Showing the first day of inoculation on Carrot Sucrose Agar media, 2. After 20th day grown culture on Carrot Sucrose Agar media, 3. First day of inoculation on Rye Agar, 4. 20th day old culture on Rye Agar

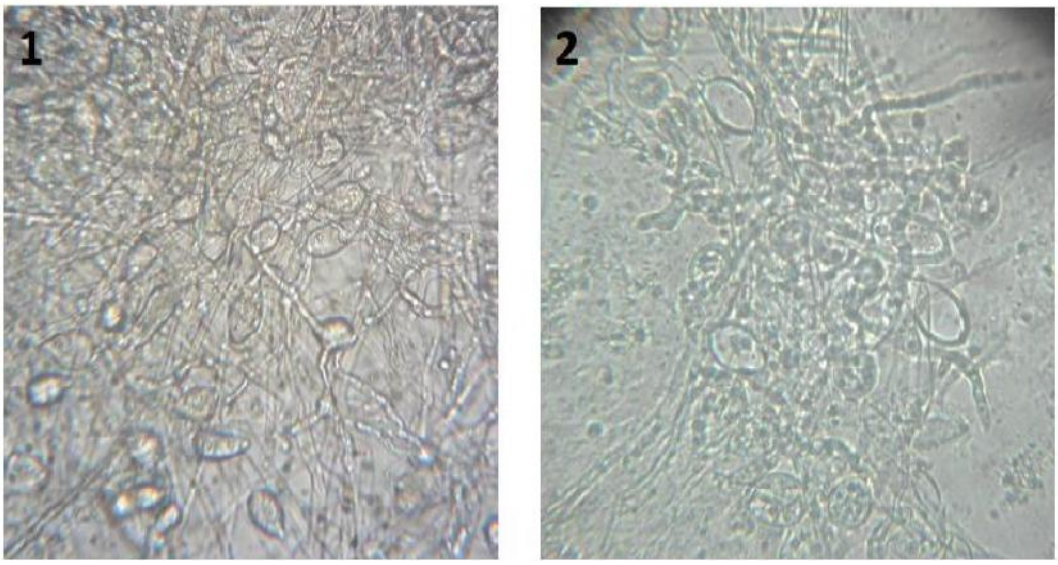

FIGURE 2: Microscopic observation (40X) - Lemon shaped sporangia and mycelia 1. Sporangia on Carrot Sucrose Agar medium 2. Sporangia on Rye Agar medium)

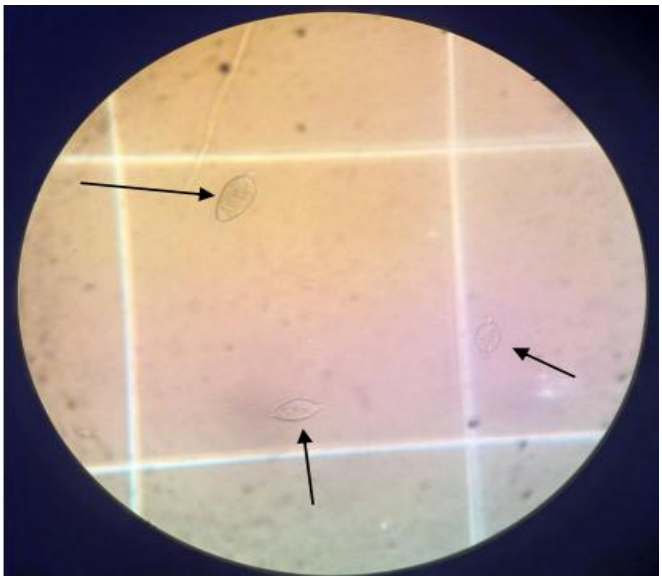

FIGURE 3: Lemon shaped sporangia on Carrot Suscrose Agar medium)

DISCUSSION & CONCLUSION

Poor growth of the fungus on different media is because of nutrient contents. For the growth and expression of fungus nutrient content is very important. According to our experiment carbohydrates are very necessary for the fungal growth as well as sporulation. Although Rye Agar media and Carrot Sucrose Agar media are the very important for the growth of the *Phytophthora infestans.* But the Rye seeds are not easily availability to everyone and ingredients for the Carrot Sucrose Agar media are easily available. For the molecular level study of the pathogen easily available media are required.

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