

PHYSIOLOGICAL STUDIES OF *Alternaria helianthi* (Hansf.) Tubaki and Nishihara, THE AGENT OF BLIGHT OF SUNFLOWER*

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SUMMARY

Attempts have been made to find a suitable medium for mass multiplication of *Alternaria helianthi*, a slow-growing fungus on potato dextrose agar. The present study revealed that natural media are better for growth and sporulation than synthetic and semi-synthetic media. For easy isolation of the fungus from diseased specimens, sunflower leaf plated on water agar and sunflower stem bits were found to be good medium sources. Sunflower leaf agar with 200 g of sunflower leaves per litre of medium without dextrose was found to be an excellent medium for mass multiplication of *Alternaria helianthi* followed by oatmeal agar and Czapek's agar medium. Colony characters varied on different media tested and formation of microcyclic conidia was observed on parent conidia in the media which contained sucrose as a carbohydrate source.

Key words: *Alternaria helianthi*, sunflower, media, mass multiplication.

INTRODUCTION

Alternaria helianthi (Hansf.) Tubaki and Nishihara, the causal agent of sunflower leaf blight, is one of the major diseases that affects yield of sunflower. Unlike other *Alternaria* spp., *A. helianthi* is a very slow-growing fungus on potato dextrose agar medium (Narain and Saksena, 1973; Anahosur, 1978; Shane *et al.*, 1981; Herr and Lipps, 1982). Hence, there is every need to determine a suitable medium for good growth and sporulation of *A. helianthi*. The present paper reports the physiological studies of *A. helianthi* on different solid and liquid media so that a suitable medium can be located which will be cheap and easy to prepare.

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Table 1: Growth of *Alternaria helianthi* on potato dextrose agar, leaf plated on water agar and sunflower stem bits

| Medium | Mycelial growth after 7 days | Number spores per ml | Colony characters |
|-------------------------------------|------------------------------|----------------------|--------------------------------------|
| Potato dextrose agar | 3.00 mm | 0.01x10 ⁵ | Dark green colony and poor growth |
| Sunflower leaf plated on water agar | Whole leaf covered | 1.00x10 ⁵ | Good growth and abundant sporulation |
| Sunflower stem bits | All the material was covered | 5.00x10 ⁵ | Good growth and abundant sporulation |

Table 2: Growth and sporulation of *Alternaria helianthi* on different solid media

| Medium | Mycelial growth (mm) | | Number of spores per ml (21 days) | Colony characters |
|--|----------------------|---------|-----------------------------------|---|
| | 7 days | 14 days | | |
| Czapek's agar | 6.38 | 16.38 | 32.00 | 5.00x10 ⁵ Greyish-green colony, powdery whitish mycelial growth underneath |
| Potato agar | 2.88 | 6.25 | 12.88 | 0.10x10 ⁵ Whitish fan-like mycelial growth |
| Potato dextrose agar | 3.05 | 7.25 | 14.38 | 0.10x10 ⁵ Dark-green colony fan like |
| Sunflower leaf extract agar without dextrose | 21.38 | 43.25 | 62.38 | 6.00x10 ⁵ Grey well-developed powdery colony |
| Nutrient agar | 4.62 | 10.55 | 17.50 | nil Pinkish colony, poor growth |
| Oat meal agar | 15.89 | 30.75 | 54.38 | 6.00x10 ⁵ Dark-green colony, good growth and sporulation |
| Barney's agar | 8.50 | 15.62 | 30.62 | 0.03x10 ⁵ Light-yellow colony, poor growth, fan like |
| Harne and Mitter's medium | 9.68 | 17.15 | 39.75 | 0.05x10 ⁵ Light-yellow colony, fan-like mycelial growth, poor sporulation |
| Richard's medium | 15.50 | 33.00 | 54.50 | 0.50x10 ⁵ Light-yellow colony with brownish yellow centre, poor sporulation and good growth |
| SEm ± | 2.25 | 1.59 | 1.34 | |
| CD (P=0.05) | 4.50 | 3.18 | 2.78 | |

MATERIALS AND METHODS

Growth of *A. helianthi* was tested on eleven solid and liquid media. The pH of the media was adjusted to 7.0 with 1N sodium hydroxide / hydrochloric acid before autoclaving. Sterilized medium (15 ml) was poured in each plate and allowed to cool at room temperature. Later, the plates were inoculated by placing agar disks of 5 mm diameter carrying the pathogen at the centre of each plate. Colony growth of *A. helianthi* was measured as radial growth on solid media. On liquid media, dry weight of the mycelial mat was recorded. Sporulation on solid media was measured by removing disk of 7 mm size from actively growing fungus and keeping them in a test tube holding 5 ml of water. Spore suspension was prepared and spore counts were made with the help of haemocytometer. For liquid media, the average number of spores from three drops under low-power objective (10x) of a microscope was counted and sporulation was graded into the following 5 categories.

| | |
|-----------------|--------------------|
| Nil | - Absent (-) |
| 1-10 spores | - Poor (+) |
| 11-20 spores | - Fair (++) |
| 21-30 spores | - Good (+++) |
| Above 30 spores | - Excellent (++++) |

The experiment was conducted in a completely randomized design replicated four times. For standardization of basal medium, a study was made to establish the optimum amount of sunflower leaf required per litre of sunflower leaf extract medium without dextrose.

RESULTS AND DISCUSSION

The physiological and nutritional needs of the fungus were studied preliminarily by culturing *A. helianthi* on sunflower stem bits, potato dextrose agar and sunflower leaf plated on water agar. The results given in Table 1 show that leaf plated on water agar and sunflower stem bits were good sources for culturing *A. helianthi* from the diseased specimens. The radial growth and sporulation of the fungus was significantly superior on sunflower leaf agar without dextrose over the rest of the media tested (Table 2). Richard's medium and oatmeal agar medium were on par with each other followed by Czapek's agar. The colony characters on different media also varied (Table 2). Microcyclic conidia were produced over parent conidia in Richard's medium. Deformation of conidia was observed on Harne and Mitter's medium and nutrient agar in the form of constriction of conidial cells on the former and bulging of conidial cells on the later.

Among the liquid media tested, potato dextrose extract and sunflower leaf extract exhibited good growth of *A. helianthi* and they were on par with each other while sporulation was significantly higher on sunflower leaf extract. Smallest

Table 3: Growth and sporulation of *Alternaria helianthi* on different liquid media

| Medium | Mycelial dry weight (g) (21 days) | Sporulation grade (21 days) | Colony characters |
|--------------------------|-----------------------------------|-----------------------------|---|
| Potato dextrose | 0.27 | +++ | Green colony, mat-like growth |
| Potato broth | 0.03 | + | Brown colour colony, poor growth |
| Sunflower leaf extract | 0.27 | ++++ | Dark-brown colony, good growth |
| Harne and Mitter's broth | 0.08 | ++ | Green colony |
| Barne's broth | 0.02 | + | Poor fan-like whitish growth |
| Czapek's broth | 0.20 | +++ | Dark-green colony, good growth |
| Nutrient broth | 0.02 | + | Pink colony, poor growth |
| Oat meal broth | 0.15 | +++ | Dark-green spores, whitish mycelium |
| Richard's solution | 0.23 | ++++ | Whitish mycelium, brownish green spores |
| SEm ± | 0.01 | | |
| CD (P=0.05) | 0.06 | | |

Table 4: Effect of quantity of sunflower leaf per litre of sunflower leaf extract without dextrose on the mycelial growth and sporulation of *A. helianthi*

| Content of leaf per litre of the medium | Mycelial growth (mm) | | | Number of spores per ml (21 days) |
|---|----------------------|-------|-------|-----------------------------------|
| 50 g | 17.30 | 32.50 | 61.63 | 0.83x10 ⁵ |
| 100 g | 19.75 | 32.25 | 61.88 | 1.50x10 ⁵ |
| 150 g | 14.25 | 29.25 | 57.75 | 3.50x10 ⁵ |
| 200 g | 13.38 | 28.63 | 57.75 | 7.50x10 ⁵ |
| 250 g | 12.38 | 29.38 | 55.18 | 6.80x10 ⁵ |
| 300 g | 10.75 | 23.13 | 43.88 | 5.80x10 ⁵ |
| 350 g | 10.00 | 23.25 | 43.25 | 4.30x10 ⁵ |
| SEm ± | 0.89 | 0.77 | 1.26 | |
| CD (P=0.05) | 3.64 | 3.13 | 5.14 | |

growth was observed on nutrient broth, Barney's broth and potato broth (Table 3). The above results clearly show that the sunflower leaf extract without dextrose was better for growth and sporulation of *A. helianthi*. Although the other media like oat meal agar and Czapek's agar were also on par with sunflower leaf extract without dextrose, the later was preferred because it is cheap and easy to prepare.

Standardization of sunflower leaf per litre of the sunflower leaf extract medium without dextrose showed that 150 g and 200 g per litre were at par with regard to the radial growth of the mycelium while the sporulation was less intensive with 150 g than 200 g per litre of the medium. The radial growth of the mycelium was more intensive in 50 g and 100 g per litre with poor sporulation while 300 g and 350 g supported good sporulation with poor radial growth. Hence 200 g of sunflower leaf per litre of the sunflower leaf extract medium without dextrose was optimum for the growth and sporulation of *A. helianthi* (Table 4). Poor growth of *Alternaria helianthi* on potato dextrose agar was also reported by several workers (Allen *et al.*, 1983a; Aponte *et al.*, 1988; Shane *et al.*, 1981 and Mukewar *et al.*, 1974). Alen *et al.*, (1983b) reported the formation of microcyclic conidia on sunflower pollen. However this is the first report indicating sunflower leaf extract without dextrose as a better medium for mass multiplication of *Alternaria helianthi*. The results also clearly show that *A. helianthi* is a low-sugar loving fungus and hence its growth on potato dextrose agar and sunflower leaf extract agar enriched with dextrose was poor.

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ESTUDIOS FISIOLÓGICOS DE *Alternaria helianthi* (Hansf.) Tubaki y Nishihara, EL INCITANTE DE LA MANCHA DE GIRASOL

RESUMEN

Se hicieron intentos para encontrar un medio adecuado para la multiplicación en masa de *Alternaria helianthi* un hongo de crecimiento lento sobre agar de dextrosa de patata. El presente estudio reveló que los medios naturales son mejores para crecimiento y esporulación que los medios sintéticos y semi-sintéticos. Para un aislamiento fácil del hongo a partir de muestras con la enfermedad, una hoja de girasol y trozos de tallo se comportaron como buenas fuentes para medios de crecimiento. La hoja de girasol en agar con 200 g de hojas por litro de medio sin dextrosa fué un excelente medio para la multiplicación en masa de *Alternaria helianthi* seguida por torta de avena en agar y el medio de agar zapek. Las características de las colonias variaron con los diferentes medios testados y la formación de conidias microcíclicas fué observada en las conidias parentales en el medio que contenía sucrosa como fuente de carbohidratos.

ETUDES PHYSIOLOGIQUES DE L'*Alternaria helianthi* (Hansf.) Tubaki et Nishihara, AGENT DE LA MALADIE DES TACHES DU TOURNESOL

RÉSUMÉ

Des essais ont été réalisés pour trouver un milieu adapté à la multiplication à grande échelle d'*Alternaria helianthi*, un champignon à faible vitesse de croissance sur substrat pomme de terre /dextrose/ agarose. Cette étude révèle que les milieux naturels sont meilleurs pour la croissance et la sporulation que les milieux synthétiques ou semi synthétiques. Pour un isolement aisé du champignon en provenance d'échantillons contaminés, la feuille de tournesol disposée sur de l'agar en solution aqueuse et les fragments de tige constituent de bonnes sources de milieux. La feuille de tournesol sur l'agar à raison de 200 g de feuilles de tournesol par litre de milieu, sans dextrose, correspond à un excellent milieu pour la multiplication à grande échelle de l'*Alternaria helianthi*, suivi par la farine d'avoine sur agarose et le milieu de Czapek sur agarose. Les caractères des colonies varient selon les milieux testés, on observe la formation de conidies microcycliques sur les conidies parentales dans le milieu contenant du saccharose comme source d'hydrate de carbone.