



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2022; 11(11): 388-390

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www.thepharmajournal.com

Received: 10-08-2022

Accepted: 13-09-2022

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Effect of different vitamins on growth and fructification of *Cercospora beticola* Sacc

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Abstract

Spinach (*Spinacia oleracea* L.), is an annual, herbaceous, nutritionally dense leafy vegetable belonging to family Amaranthaceae. Spinach is attacked by various fungal diseases like damping off and leaf spot. Among these, leaf spot caused by *Cercospora beticola* is one of the limiting factors in commercial cultivation of palak. Fructification study was carried on Richards Agar media impregnated with various vitamins and the growth and sporulation was recorded significantly superior with the incorporation of vitamin combination of thiamine + pyridoxine + choline, followed by thiamine + pyridoxine. Minimum growth and sporulation was observed with the addition of pyridoxine alone with no sporulation when devoid of any vitamin. The results clearly indicate that the vitamins play significant role in the fungal reproduction.

Keywords: Amaranthaceae, *Cercospora beticola*, *Spinacia oleracea* L.

Introduction

Spinach (*Spinacia oleracea* L.) an important leafy vegetable, belonging to family Amaranthaceae. Spinach is an economically important vegetable crop grown worldwide (Morelock and Correll, 2008; Correll *et al.*, 2011) [1, 2]. In addition to its economic importance, spinach is one of the faster growing vegetable crops in the US and other regions in terms of per capita consumption and is considered one of the healthiest vegetables in the human diet due to its high concentration of nutrients and health promoting compounds (Dicoteau, 2000; Morelock and Correll, 2008) [1, 2]. Nutritionally spinach is rich in vitamins such as vitamin A, B, C and E and in minerals like magnesium, manganese, calcium and besides being an excellent source of chlorophyll which aids in digestion and contains good amount of antioxidants and has one of the highest oxygen radical absorbance capacity (ORAC) value and is also known to be rich source of phyto-nutrients (Prior, 2003; Otari *et al.*, 2010) [4, 5]. Spinach is one of the most desirable green leafy vegetable because of high beta carotene content (pro vit. A) and also a good source of iron phosphorus, sodium and potassium (Dicoteau *et al.*, 2000) [3]. The agro-climatic conditions of Kashmir valley are quite conducive for spinach cultivation which also favours the development of various fungal diseases. Among the various emerging diseases, *Cercospora* leaf spot disease has assumed an alarming status resulting in huge economic losses. In Kashmir, during favourable environmental conditions especially during high precipitation the crop is attacked from its early to maturity stage by this disease resulting in severe leaf spotting, yellowing and pre-mature withering of the plant which is a major constraint in its successful cultivation. While working on cultural studies of *Cercospora* species, many researchers have found difficulty in its rapid growth and sporulation. Synthetic as well as semi synthetic artificial media amended with vitamins have been used for enhancing the fructification of the fungus. No such work was carried out in Kashmir, so the study was taken up to overcome the difficulty of fructification and slow growth of *Cercospora beticola*.

Materials and Methods

In order to ascertain which of the vitamin supports the best growth and sporulation of the fungus, three vitamins at their recommended concentrations *viz.*, thiamine (1 mg /lit.), pyridoxine (0.5 mg/lit.) and choline (5 µg /lit.) alone as well as in combination were evaluated on Richard's Agar medium. To work out the desired concentrations of vitamins, 100 mg of thiamine was dissolved in 1000 ml of distilled water to make the stock solution; from which one ml was added to 99 ml of Richard's Agar medium to get the required concentration.

Similarly, 100 mg of pyridoxine was dispensed in 2000 ml of distilled water, out of which 1ml was impregnated in 99 ml of medium to get 0.5 mg concentration. Likewise 5 mg of choline was dispensed in 1000 ml of water, which was further diluted by dissolving 1 ml of stock solution in 9 ml of water, from which 0.1 ml was pipetted out and added to 99.9 ml of medium, so as to get the required concentration. Uniform volumes of sterilized medium impregnated with various vitamins was poured into sterilized petri plates (100 mm diameter) under aseptic conditions. The petri plates were inoculated separately with uniform inoculum disc (5 mm) from 30 days old culture, cut with the aid of sterilized cork-

borer from the periphery of the fungul colony already grown for this purpose. Four replications were maintained for each treatment and incubated at 25 ± 2 °C for 30 days. Observations regarding the radial mycelial growth in all the four replications for each treatment was recorded and average of all the four replications taken as final measurement.

The sporulation for each treatment was studied by homogenizing thoroughly 5 mm dia. mycelial disc in 5 ml of sterilized water. The spore suspension thus obtained was used for measuring the sporulation for each treatment using a haemocytometer.

Table 1: Different vitamin combination for fructification of pathogen

	Treatment	Qty/litre
T ₁	Thiamine	1 mg
T ₂	Pyridoxine	0.5 mg
T ₃	Choline	5 µg
T ₄	Thiamine + Pyridoxine	1 mg + 0.5 mg
T ₅	Thiamine + Choline	1 mg + 5 µg
T ₆	Pyridoxine + choline	0.5 mg + 5 µg
T ₇	Thiamine + Pyridoxine + Choline	1 mg + 0.5 mg + 5 µg
T ₈	Check	No vitamin

Table 2: Effect of various vitamins on radial mycelial growth and fructification of *Cercospora beticola* Sacc

S. No.	Treatment	Concentration/liter	Radial mycelial growth (mm)*	Sporulation (1×10^4 spores/ml)**
1	Thiamine	1 mg	46.90 ^{cd}	0.80 ^{bc}
2	Pyridoxine	0.5 mg	42.30 ^e	0.20 ^{ef}
3	Choline	5 µg	44.36 ^e	0.40 ^{de}
4	Thiamine+ Pyridoxine	1 mg + 0.5 mg	52.50 ^b	0.80 ^{bc}
5	Thiamine + Choline	1 mg + 5 µg	49.31 ^c	1.00 ^{ab}
6	Pyridoxine + Choline	0.5 mg + 5 µg	45.42 ^d	0.60 ^{cd}
7	Thiamine + Pyridoxine + Choline	1 mg + 0.5 mg + 5 µg	56.42 ^a	1.20 ^a
8	Check	No vitamin	38.81 ^f	0.00 ^f
CD ($p \leq 0.05$)	2.74	0.20		

*Average of four replications; **Average of 20 observations; Growth and sporulation recorded 30 days after incubation; Figures with same letter(s) are statistically identical.

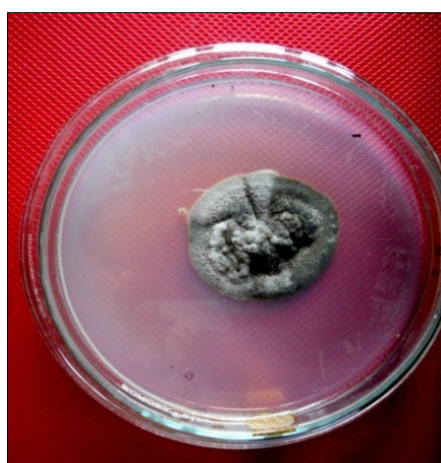


Fig 1: 45 days old fungal colony grown on Richard's agar medium amended with thiamine

Results and Discussion

Nutritional studies are important for finding the optimum requirement of nutrients for the growth and sporulation of the fungus. To determine the best vitamin which supports the optimum growth and sporulation of *C. beticola*, Richard's agar medium, the basal medium on which the pathogen was grown, was incorporated with different vitamins viz.,

thiamine, pyridoxine and choline alone as well as in combinations. The average radial mycelial growth (mm) and sporulation/ml of water for each treatment was recorded after 30 days of incubation at 25 ± 2 °C.

The data presented in Table 2; Fig. 1 reveals that the fungus can utilize a number of vitamins for its growth and sporulation. The radial mycelial growth of the fungus was best achieved on vitamin combination comprising of thiamine + pyridoxine + choline (56.42 mm) followed by thiamine + pyridoxine (52.50 mm). Vitamin combination of thiamine + choline yield radial mycelial growth of (49.31mm). Minimum growth (42.30 mm) was achieved by the addition of pyridoxine alone.

The data further reveals that the fungus gave maximum sporulation of 1.20×10^4 spores/ml of water when the basal medium was amended with vitamin combination of thiamine + pyridoxine + choline, followed by thiamine + choline (1×10^4 spores/ml) and thiamine + pyridoxine (0.80×10^4 spores/ml). No significant difference however, was observed between thiamine + pyridoxine + choline and thiamine + choline and also between thiamine + pyridoxine and thiamine + choline in supporting the sporulation of the fungus. Minimum sporulation of 0.20×10^4 spores/ml was achieved with the addition of pyridoxine alone. No sporulation was

however, obtained when the basal medium was devoid of any vitamin. Although the fungus has shown differential response to different vitamins, but the growth and sporulation was recorded significantly superior with the incorporation of vitamin combination of thiamine + pyridoxine + choline, followed by thiamine + pyridoxine. Minimum growth and sporulation was observed with the addition of pyridoxine alone with no sporulation when devoid of any vitamin. The results clearly indicate that the vitamins play significant role in the fungal reproduction, which is in accordance with the findings of Thind and Mandahar (1965) ^[6] who also reported thiamine essential for sporulation of *Cercospora beticola*.

Conclusion

Spinach is attacked by various fungal as well as bacterial diseases, among which *Cercospora* leaf spot caused by *Cercospora beticola* is most serious disease causing huge economic loss by deteriorating its quantity as well as quality. Cultural studies of the pathogen showed that the fungal growth and sporulation can be enhanced by addition of individual or combination of vitamins in Richard's agar media.

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