

# Management of *Cercospora* Leaf Spot of Indian Spinach (*Basella alba* L.) with BAU Bio-fungicide and a Plant Growth Promoting Hormone

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**Abstract** *Trichoderma* based BAU-biofungicide, chemical Carbendazim and a synthetic plant growth promoting (PGP) hormone have been used to study their effect on *Cercospora* leaf spot of Indian spinach. Number of leaf, number of infected leaf, disease incidence, disease severity, area under disease progress curve (AUDPC), plant height and plant weight were measured and significant variations was found against different treatment combinations. Among the treatment combinations, seed treating with Carbendazim followed by foliar spray with Carbendazim, seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, only foliar spray with Carbendazim and only spray of PGP hormone significantly reduced disease incidence and severity with increasing of plant height and weight. Interestingly, foliar application of PGP hormone reduces disease incidence and severity by 58.38% and 63.8% in both the variety of Indian spinach, respectively over control, whereas; in both the variety disease incidence and severity is reduced by seed treatment with Carbendazim followed by foliar spray with Carbendazim 69.72% & 77.63%; seed treatment with BAU bio-fungicide followed by foliar spray with Carbendazim 63.73% & 69.49%; foliar spray with Carbendazim 55.68% & 58.19%, respectively. Foliar application of PGP hormone along with the reducing of disease incidence and severity, also increases the leaf number by 68.62%, plant height by 54.64% and plant weight by 65.69% over control. AUDPC showed that, local variety of Indian spinach is more susceptible to *Cercospora* leaf spot than BARI spinach 1.

**Keywords** *Cercospora* Leaf Spot, Indian Spinach, PGP Hormone, BAU Bio-fungicide

## 1. Introduction

Indian spinach (*Basella alba* L.) belongs to the family

Basellaceae is an indigenous, rapidly growing, tropical leafy vegetable [1,2] commonly grown as backyard plant in the home gardens. It is originated from India or Indonesia [3], however; it is also popular in tropical and subtropical region including Asia, America, Africa, Madagascar etc. [2]. The vegetable has other interesting common names in different region like Ceylon spinach, Malabar spinach, saan choy (Chinese), mongtoi (Vietnamese), alugbati (Philippines), puisaag (Bengali), remayong (Malay) etc. Nutritionally, it is low content of carbohydrate and fat but holds good amount of vitamins (Vitamin-A -3250IU/100g), minerals Iron 1.4%, Calcium 0.15%, and Antioxidants ( $\beta$ -carotene, lutein, zeaxanthin) [1].

Leaves are the prime edible parts of Indian spinach vegetable. Since, the yield or production data of this vegetable is not available in Bangladesh; it is assumed that, a vast amount of yield is lost in terms of quantity and quality due to various constraints. Among the constraints, diseases especially *Cercospora* leaf spot plays a vital role for the qualitative loss of this vegetable. *Cercospora* leaf spot is caused by *Cercospora beticola* which affect mainly on leaf. The typical symptoms appear as circular to oval shaped, purple color pinhead spots with a necrotic gray centre surrounded by a purple to brown border [4]. Due to *Cercospora* leaf spot disease, photosynthetic process is disturbed and leaves becomes deformed resulting weakens plant, premature defoliation which ultimately lowers the yield and market value.

The causal agent *Cercospora* mainly is seed born, however; the pathogen is also able to survive for at least one year in plant debris and soil also. Primarily their spores are dispersed by wind and is favored by prolong rainfall, high relative humidity and 25°C to 35°C temperature [4]. The fungus *Cercospora* also causes numerous economically relevant plant diseases on other leafy vegetable like Palong [5], egg-plant [6], legume crop like sesame, groundnut [7,8] and also in mungbean [9] where the yield losses were recorded up to 58% [10].

To reduce the market loss of the vegetable, peoples usually use chemical for management of the disease. Since it is leafy vegetable, farmers also use some synthetic PGP hormone to improve its production. In our study test chemicals chosen were Carbendazim and Foliar plus growth hormone to show the treatment effect on various growth parameter as well as *Cercospora* leaf spot disease of Indian spinach. Carbendazim is widely known as Benzimidazole compound owing to the excellent systemic control of much important plant diseases [11]. Other way “Foliar plus” is foliar liquid which basically contain auxin and gibberellic acid (GA3) (200ppm), including some other macro and micro nutrients like nitrogen, muriate of potash, zinc, calcium, magnesium, iron, manganese etc.

Plants by nature generate various types of hormones, which include auxins, gibberellins (GA), abscisic acid (ABA), cytokinins (CK), salicylic acid (SA), ethylene (ET), jasmonates (JA), brassinosteroids (BR) and peptide hormones, play vital roles not only in growth and developmental processes but also in biotic and abiotic stress responses in plants [12]. Phytohormone also changes its level of secretion during pathogenic attack of plant [13,14].

As chemicals have undescribed contribution on agriculture to control diseases and yield improvement but indiscriminate and frequent use of this chemical ultimately creates serious health hazards by entering in food web. So, now days, plant pathologist give an attention on sustainable and eco-friendly agricultural practices for the management of plant diseases. In Bangladesh few approaches have been taken to evaluate efficacy of *Trichoderma* based bio-fungicide and PGP hormone against plant disease development. In the light of above background, present investigate has been taken so far for the first time in order to evaluate a *Trichoderma* based BAU bio-fungicide and plant growth promoting (PGP) hormone against *Cercospora* leaf spot (CLS) disease of Indian spinach.

## 2. Materials and Methods

### 2.1. Experimental Site and Design

Seeds of two variety of Indian spinach BARI spinach 1 and local variety were collected from Bangladesh Agricultural Research Institute and local market, respectively. The collected seeds were sown at experimental field of Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from May to July 2008. Seeds were sown on 27 may 2008 and plants were harvested on 28 July 2008. The experiment was laid out in randomized complete block design with three replications.

### 2.2. Experimental Materials and Treatments

*Trichoderma* based BAU bio-fungicide, chemical Carbendazim and a PGP hormone (Foliar Plus) were used by

eight different treatment combination to manage the *Cercospora* leaf spot disease as following combinations: T<sub>0</sub>: control, T<sub>1</sub>: seed coating with BAU bio-fungicide followed by foliar spray with suspension, T<sub>2</sub>: seed treating with Carbendazim followed by foliar spray with Carbendazim, T<sub>3</sub>: seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, T<sub>4</sub>: seed treating with Carbendazim, T<sub>5</sub>: seed coating with BAU bio-fungicide, T<sub>6</sub>: foliar spray with Carbendazim and T<sub>7</sub>: foliar spray with PGP hormone.

### 2.3. Seed Coating and Application of BAU Bio-Fungicide

Seeds were coated with BAU bio-fungicide @ 1:40 w/w following the published method [15] by moistening the seed with rice forth. The seeds were thoroughly mixed with the BAU bio-fungicide by shaking the petridishes and then seeds were placed in a cool and dry place for drying until sowing in the field. Spray suspension of the bio-fungicide were made by scrapping the spores from cultured *Trichoderma* pure culture and diluted in sterile distilled water. The numbers of spores were counted by using a haemocytometer and finally 10<sup>7</sup> spores/ml was used to spray. The spore suspension was sprayed three times at 15, 30 and 45 days after sowing.

### 2.4. Seed Treatment with Carbendazim and Foliar Spray of Chemicals

Seeds were treated with Carbendazim by using sterilized petridishes where a little sterilized distilled water was used to moisten the chemical. Foliar spray solution of the fungicide was prepared by dissolving the required amount in water to make the final concentration of 0.1%. Plant growth promoting hormone solution was also prepared by dissolving 10 ml/10 L water. Both the solutions were sprayed three times at 15, 30 and 45 days after sowing.

### 2.5. Scoring of Disease

The data on number of leaf, number of infected leaf, number of spot on leaf were recorded up to harvest by visual observation at 21, 27, 33, 39, 45 and 51 days after sowing. Disease incidence and severity was measured into five categories following a disease grading scale where 0= No visible symptom; 1= 1 to 10% leaf area affected; 2=11 to 20% leaf area affected; 3= 21 to 50% leaf area affected; 4= 51 to 80% leaf area affected and 5= >80% leaf area affected [16]. The disease intensity accomplished with percent disease index was calculated by using following formula [17].

$$\text{Disease Incidence(\%)} = \frac{\text{Total number of leaf/plant}}{\text{Number of infected leaf /plant}} \times 100$$

$$\text{Disease Severity (\%)} = \frac{\text{Sum of all disease rating}}{\text{Total number of rating} \times \text{maximum disease grade}} \times 100$$

We have also measured area under disease progress curve from PSI and calculated according with the following formula [18].

$$\text{AUDPC} = \sum_{i=1}^{n-1} 0.5[(X_i + X_{i+1})(t_{i+1} - t_i)]$$

Where, n =total number assessment time, t = time of  $i^{\text{th}}$  assessment,  $X_i$  = percentage of disease severity at  $i^{\text{th}}$  assessment.

### 2.6. Analysis of Data

Data on number of leaf, number of infected leaf, disease incidence, disease severity and value of AUDPC were subjected to analysis of variance, to evaluate the efficacy of different treatment combination, mean separations were done using Duncan's Multiple Range Test (DMRT).

## 3. Results

From our present study, significant variations were found among the treatment combinations in order to increase the plant growth parameter along with reducing the diseases intensity of Indian spinach.

### 3.1. Number of Leaf per Plant

Since Indian spinach is a leafy vegetable, hence; number of leaf is an important parameter to observe the effect of different treatments. At the beginning of the treatment application, more or less equal number of leaf per plant was observed, however; with the time, after each application of PGP hormone, leaf number increased considerably in compare with control and other treatments. Between the two varieties used, BARI spinach 1 showed higher number of leaf in compare with local variety. BARI spinach 1 treated with PGP hormone produced 8.00 to 41.00 number of leaf per plant where under the same treatment local variety produced 7.00 to 34.33 number of leaf per plant. While the lowest number of leaves in BARI spinach 18.00 to 24.00 and in local variety 7.33 to 20.67 were observed with untreated control (Figure 1 A, B).

### 3.2. Number of Infected Leaf per Plant

Number of infected leaf per plant was found to reduce by the application of Carbendazim and PGP hormone. The highest numbers of infected leaf (4.33 to 11.00) were observed in local variety without any treatment. Similar kind

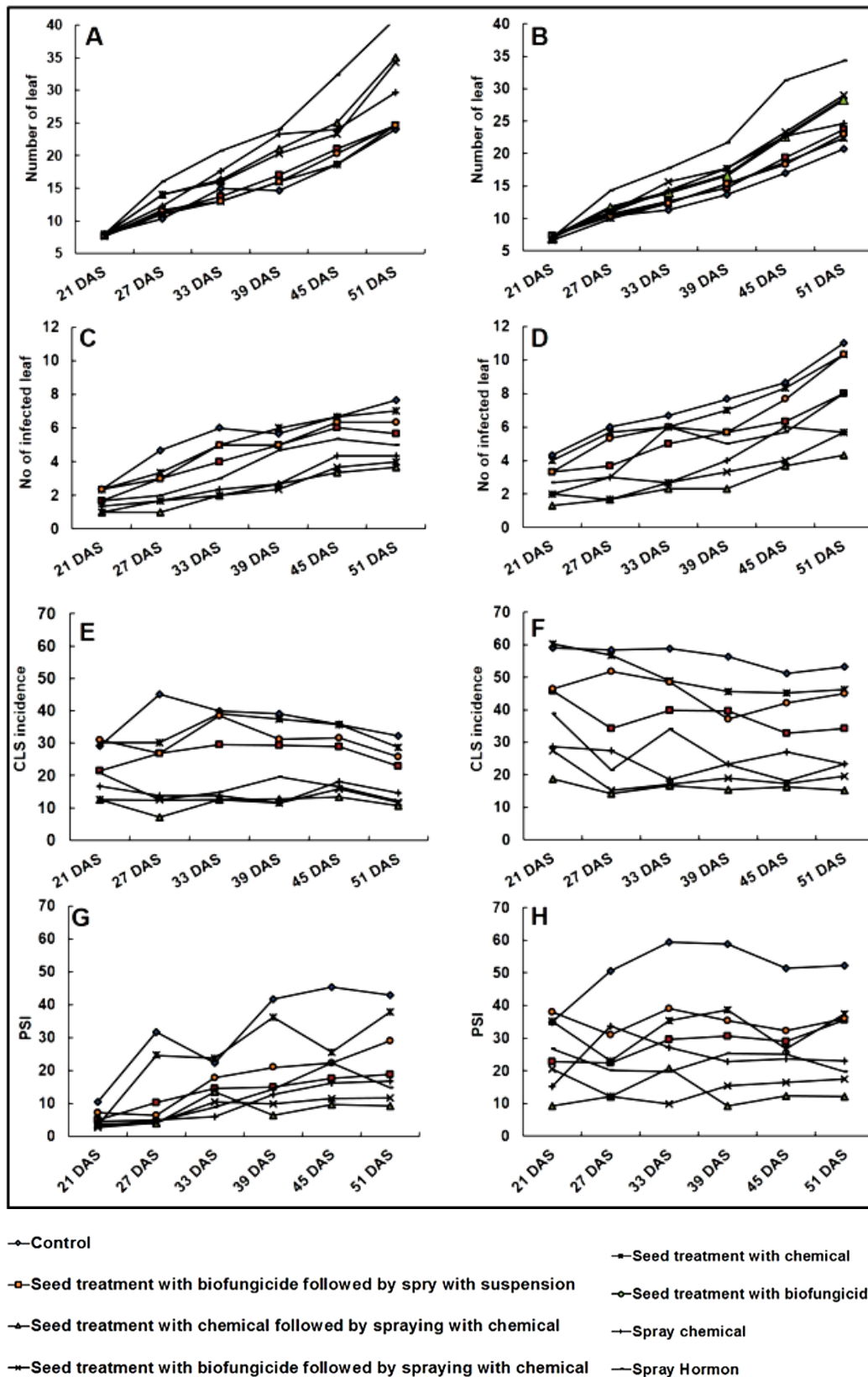
of observation was also found with seed treatment with Carbendazim (4.00 to 10.33) and seed treatment with BAU-bio-fungicide (3.33 to 10.33). In BARI spinach 1, lowest number of infected leaf was found in seed treatment with Carbendazim followed by foliar spray with Carbendazim (1.00 to 3.67), statistical similar result were also found in seed treatment with BAU bio-fungicide followed by foliar spray with Carbendazim (1.00 to 4.00), foliar spray with Carbendazim (1.33 to 4.33) and foliar spray with PGP hormone (1.67 to 5.0) (Figure 1 C, D).

### 3.3. Disease Incidence

Disease incidence showed significant difference among the cultivars and treatment effect. Six inspections on percent disease incidence were visually assessed at 21, 27, 33, 39, 45 and 51 days after sowing. Maximum decrease of disease incidence was found in BARI spinach 1 in compare with local variety. The highest disease incidence (58.90 to 53.19) was observed in local variety without giving any treatment which was statistically similar with seed treatment with Carbendazim (60.30 to 46.25), seed treated with BAU bio-fungicide (46.40 to 44.93). BARI spinach 1 variety with foliar application of Carbendazim (16.67 to 14.62) and PGP hormone (20.83 to 12.18) provides minimum disease incidence (Figure 1 E, F).

### 3.4. Disease Severity

Among the various treatments, statistical significant result is found with seed treatment Carbendazim followed by foliar spray of Carbendazim, seed treatment with BAU bio-fungicide followed by foliar spray of Carbendazim, foliar spray of Carbendazim and foliar spray of PGP hormone in each variety. The greater disease severity was observed in local variety (35.00 to 52.20) without any treatment followed by seed treated with Carbendazim (35.24 to 37.40), seed coating with BAU bio-fungicide (38.10 to 35.91) and the lowest was observed in BARI Spinach 1 variety by seed treatment with Carbendazim followed by spray with Carbendazim (3.73 to 9.26), seed treated with BAU bio-fungicide followed by spray with Carbendazim (2.74 to 11.60), foliar spray with Carbendazim (4.44 to 16.74) and foliar spray with PGP hormone (3.33 to 14.67) in BARI spinach 1 variety (Figure 1 G, H).



**Figure 1.** Effect of Carbendazim, BAU bio-fungicide and PGP hormone in different combinations on number of leaf (A. BARI spinach 1, B. Local variety), number of infected leaf (C. BARI spinach 1 D. Local variety) Disease incidence (E. BARI spinach 1 F. Local variety) and percent disease index (G. BARI spinach 1 H. Local variety)

**Table 1.** AUDPC of *Cercospora* leaf spot of Indian Spinach in response to different treatments applied

Treatment	AUDPC (%)	
	BARI Spinach 1	Local variety
Control	1212	1969
Seed coating with BAU bio-fungicide followed by foliar spray with suspension	502.6	1065
Seed treated with Carbendazim followed by foliar spray with Carbendazim	293.4	486.2
Seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim	312.4	582.6
Seed treating with Carbendazim	926.9	1245
Seed coating with BAU bio-fungicide	624.0	1355
Foliar spray with Carbendazim	367.0	937.1
Foliar spray with PGP hormone	424.6	886.6
Mean	582.79	1065.81
CV (%)	9.13	
LSD(0.05)	125.5	

**Table 2.** Effect of different treatment combinations on plant height and weight of Indian Spinach

Treatment	BARI Spinach 1		Local variety	
	Height(cm)	Weight (g)	Height (cm)	Weight (g)
Control	68.33 cdef	143.3 cd	50 f	133.3 d
Seed coating with BAU bio-fungicide followed by foliar spray with suspension	79.00 bc	170.7 bcd	56.67 def	160.0 bcd
Seed treated with Carbendazim followed by foliar spray with Carbendazim	92.67 ab	180.0 bcd	64.33 cdef	171.7 bcd
Seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim	100.7 a	183.3 bcd	61.33 cdef	170.0 bcd
Seed treating with Carbendazim	74.67 bcd	145.0 cd	50.17 f	151.7 cd
Seed coating with BAU bio-fungicide	79.00 bc	160.0 bcd	52.67 ef	155.0 cd
Foliar spray with Carbendazim	99.67 a	200.0 abc	59.67 cdef	163.3 bcd
Foliar spray with PGP hormone	111.0 a	240.0 a	72.00 cde	218.3 ab
CV (%)	15.22	17.87	15.22	17.87
LSD	18.58	51.14	18.58	51.14

\*Significant at 5% level of probability. Values having same letters within the same column do not differ significantly

### 3.5. Area under the Disease Progress Curve

Area under disease progress curve (AUDPC) was calculated to assess the disease severity of two Indian spinach varieties with eight different treatment combinations in order to find out the effectiveness of treatment mixture for managing CLS disease. The highest AUDPC (1212) was calculated from untreated control of BARI spinach 1, while the lowest AUDPC (293.4) estimated from seed treated with Carbendazim following the foliar spray with Carbendazim. The maximum AUDPC (1969) was calculated in local variety with control and minimum (486.2) in the plant with seed treatment with Carbendazim followed by spraying with Carbendazim. Foliar spray with PGP hormone also gave the lower (424.6 and 886.6) AUDPC (Table 1).

### 3.6. Plant Height and Plant Weight

*In vivo* studies of different treatment on plant height and weight showed that foliar spray with PGP hormone (111.0

cm, 240gm) was the most effective in relation to the control and other treatment in BARI Spinach 1. Next best result in the same variety was found with foliar spray of Carbendazim (99.67cm, 200gm.), seed treatment BAU bio-fungicide followed by foliar spray of Carbendazim (100.7cm, 183.3gm) and seed treatment with Carbendazim followed by foliar spray of Carbendazim (92.67cm, 180gm). Minimum plant height and weight was found (50.0cm, 133.3gm) in local variety with control followed by Seed treated with Carbendazim (50.17cm, 151.7gm) and seed coating with BAU bio-fungicide (52.67cm, 155.0gm) (Table 2).

## 4. Discussion

The study was carried out in two Indian spinach varieties to evaluate the action of *Trichoderma* based bio-fungicide and “foliar plus” PGP hormone against *Cercospora* causing leaf spot disease in order to increase plant growth parameters

along with reduce disease severity. Among the eight different treatment combination, PGP hormonal treatment showed tremendous effect on growth parameters, increasing leaf number as 70%, plant height as 54.64% and weight as 65.58% accompanied by reducing disease incidence and severity. Similar to our findings, increasing height of the plant, collar diameter, number of leaf with the application of PGP hormone also reported by several scientist [19]. Due to the application of PGP hormone, chlorophyll content is augmented which lead to the greater leaf production [20] as well auxin hormone maintains apical dominance of plant and promotes stem elongation and cell division. GA (gibberellic acid) stopping the expression of DELLA protein which act as negative regulator of plant growth and enhance seed germination, shoot elongation and modulates floral biology [21]. *Trichoderma* based BAU bio-fungicide also showed the positive effect on plant growth parameters, however; the effect was less in compare with PGP hormone and Carbendazim treatment. Several studies also reported that plant height and weight increased with BAU bio-fungicide in different vegetables [22] and in this study, BAU bio-fungicide (seed treatment followed by spray) averagely increased plant height from 59.75 to 67.84 cm in both varieties. In lettuce, Yedricula cultivar with the application of *Trichoderma* in per peat number of leaf calculated 3.8 to 4.3, hypocotyls length measured 5 to 8.5 cm, and seedling fresh weight found 3.8 to 4.3 gm at 15 gm/m<sup>2</sup> dosages and root weight resulted 0.18 to 0.28 gm at 10gm/m<sup>2</sup> dosages [23].

Our study also revealed that PGP hormone also plays an important role against disease development in foliage which is nearer to Carbendazim treatment effect. Foliar spray of PGP hormone and seed treatment followed by spray bio-fungicide suspension reduced the disease incidence and severity by 58.38% and 63.8%, respectively. For plant growth and development auxin encourage the expression of three group of genes Aux/IAA, GH3 family and small auxin-up RNA (SAUR) family [24]. Among these three families GH3 has been shown defense mechanism in *Arabidopsis* and rice as well during pathogen infection GH3.5 response as bio-functional modulator together with auxin and SA signaling [25]. GA also play plant defensive role to biotrophs or necrotrophs by dual regulating and balancing SA (salicylic acid) and JA/Et mediated signaling pathways [21]. PGP hormones are plant growth regulator acted as an elicitor [20] triggers secretion of secondary metabolites in leaf and hinder the advancement of pathogen [26]. Phenolics compound like garlic acid, Chlorogenic acid, Vanillic acid, Caffeic acid and Phytoalexins are most of the secondary metabolic plant induce themselves for defensive action against pathogen [20, 26]. Polyphenolic compounds as fungitoxics show negative impact on germination of spores of *Cercospora* in tomato and sugar beet [26]. PGRs play an importance function by protecting cell membrane fluidity and integrity. Enzymatic and non-enzymatic machinery are mediate by plant growth hormone resulting the prevention of cell membrane damage by Reactive oxygen

Species [27]. Foliar spray of PGP hormones also found to effective to enhance total soluble protein (TSP), malondialdehyde (MD), reducing power, DPPH free radical scavenging potential in spinach which signaling defense mechanism in plant [20]. Once fungi detected by *Trichoderma* its start to go towards them [28] attach to the host and form appressorium like structure around the hosts secrete fungitoxic and peptibol antibiotic [29] through which build a parasitism relationship with target fungus and causes dissolution of the cell walls [30].

We have used Carbendazim as positive control against the *Cercospora* leaf spot of two Indian spinach varieties. Seed treatment along with foliar spray with Carbendazim reduced the disease incidence 69.72% and severity 77.63% over all other treatments. Benzimidazole fungicides are specific inhibitor of microtubules assembly connect heterodimeric subunit, the tubulin molecule [11]. Management of *Cercospora* blight disease with Carbendazim has also gave the lowest disease incidence and maximum seed yield in groundnut and Okra [8, 31].

## 5. Conclusions

Foliar application of selected PGP hormone and Carbendazim play statistically significant role to control leaf spot disease of Indian spinach along with the increasing of number of leaf, plant height and weight. Although, *Trichoderma* based BAU-fungicide increased the plant growth along with reducing the disease severity, the effect is lower than PGP hormone. However, PGP hormone found to be the most potent tools to obtain the vigorous Indian spinach with less loss of market value. However, to reduce the negative effect of the chemicals on environment, further study needs with *Trichoderma* based bio-fungicide along with PGP hormone to elucidate the best combination for *Cercospora* leaf spot management.

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