Eco. Env. & Cons. 28 (4) : 2022; pp. (2030-2033)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i04.056

Eco-friendly Approaches against *Alternaria alternata* Causing Leaf Spot of Stevia (*Stevia rebaudiana*)

Sushma S. M. G. [1]**, Naveen K. R.** [1] **and Dharani U.** [1]

Department of Plant Pathology, Naini Agricultural Institute, SHUATS, Prayagraj 211 007, U.P., India

(Received 23 April, 2022; Accepted 18 June, 2022)

ABSTRACT

Stevia (*Stevia rebaudiana*) is a small perennial and herbaceous shrub that originated from South America and belongs to the *Asteraceae* family. Stevia is also known "sugar substitute, sweet weed, candy leaf and sweet herb" and also a chief source of vitamin 'C'. Stevia leaves are high in folic acid and rich in carbohydrates and reducing sugars estimated to be 300 times sweeter than sugarcane. Alternaria leaf spot caused by *Alternaria alternata* is the most common disease in stevia, An experimental trial was taken up to evaluate the efficacy of selected eco-friendly treatments on the growth parameters in stevia over *Alternaria alternata* at Central Research Farm, Division of Plant Pathology, Naini Agricultural Institute, U.P. Maximum plant height (50.40 cm), Maximum number of branches (23) and minimum disease intensity (22.79%) are recorded in the treatment combination of T_8 - Microalgae + neem cake + vermicompost followed by T_6 – Microalgae + vermicompost.

Key words : *Alternaria alternata, FYM, Mycorrhizae, Microalgae, Neem cake, Stevia rebaudiana, Vermicompost.*

Introduction

Stevia (*Stevia rebudiana*) is an herbaceous perennial shrub that belongs to the family Asteraceae recognized as a unique crop for its sweetening property, it is popularly known as "candy leaves, non-caloric sweet plant, sugar substitute, sweet weed, honey leaf and sweet herb". Stevia is indigenous to Brazil and native to Paraguay where it grows in sandy soils nearby streams (Dhruva *et al.,* 2014) and is propagated through seeds or cuttings. The leaves of stevia are 10-15 times sweeter than sucrose; possessing natural sweetening agents it can be used as diabetic-friendly (Singh *et al.,* 2015). The unique sweetening property of stevia is due to the presence of sweet-tasting and low calories di-terpene-stevioglysides (SG's), the leaf extract has proven to benon-toxic on mankind and has been used conventionally in many remedies. Some sweeteners such as stevioside and rebaudioside are present in concentrations of nearly about 3-10% to 1-3% of dry leaf matter (Dushyanth *et al.,* 2014). In addition, to unique sweetening property, the leaves are chief sources of vital minerals like potassium, calcium, sodium, zinc and iron essential for various metabolic activities of mankind (Arab *et al.,* 2000). Besides these it also possesses anti-fungal, anti-bacterial properties and also helps in preventing dental caries (Yadav *et al.,* 2010). The sustainable cultivation of *Stevia rebaudiana* is ruined by various biotic and abiotic agents. Among the biotic factors phytopathogens *viz Septoria stevia, Fusarium Spp, Alternaria alternata, Rhizoctonia solani* are very common. Out of these Alternaria leaf spot caused by *Alternaria*

([1]Master Research Scholar)

alternata is the most destructive pathogen which results in unbearable losses in both quality and quantity of the produce. To reducethese impactand to support the sustainable practice several management practices have been emerged, but these are of chemical origin and are hazardous to the environment and mankind. So, considering the above facts a research experiment was conducted to evaluate the effects of eco-friendly combinations on growth parameters against Alternaria leaf spot of stevia.

Materials and Methods

The present experimental study was conducted at CRF, Division of Plant Pathology, Naini Agricultural Institute, U.P. during the *Rabi* season in 2021 with a randomised block design.

Collection of disease sample:

The infected leaves exhibiting typical symptoms are fetched from the standing stevia crop and brought to the laboratory for further investigation.

Isolation of the pathogen

The leaves from infected plants were identified and selected. The spotted area on the leaf surface is identified and cut into small pieces of about 4-6 mm, these pieces are washed with freshwater followed by running tap water. 20 ml (approx.) of sterilized molten warm PDA media was poured into sterile distilled plates aseptically. The leaf pieces are further surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 30 seconds followed by washing thrice with distilled water and allowed to dry. Later, these dried pieces were carefully placed on the molten media (PDA) in invested position to ensure that the spot area contacts with the media. And these plates are incubated at 25±2 °C for 2-3 days. After, the obtained fungal mycelial growth was examined under the microscope and used for further studies.

Disease incidence (%)

The disease incidence (%) was calculated by using the formula mentioned below

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Morphology and symptomology of the pathogen

Themycelium of the pathogen is septate. Conidiophores are simple, septate, olive-brown and vary in length with solitary terminal conidia or chains (Fig. 2 & 3). Initially, the symptoms appear as a small oval dark brown necrotic sunken spot (2-18 mm dia.) found at the leaf tips, these later on forms as large necrotic patches (Fig. 1).

Results and Discussion

The results depicted in the Table 1 and Fig. 1 disclose that all the treatment combinations are statistically similar and minimised the disease incidence (%) as compared to control. Among the combinations applied T_8 – microalgae + neem cake + vermicompost significantly decreased the disease incidence (%) by 22.79 when compared to remaining. All the treatments are found to be significant over control, but among the treatments (T_4, T_7 and T_2), (T_2, T_3), (T_1, T_9 and T_5) and (T_9, T_5 and T_6) are found to be non-significant. Similar results were reported by (Maiti *et al.*, 2007) with the application of microalgae.

Table 1. Effect of bioresources on plant height of *Stevia rebaudiana*

Treatments		Plant height (cm)	
		Before application	60 DAT
T_0	Control	16.25	25.83
T_1	Microalgae	19.06	37.40
T_3	Neem cake	16.10	32.20
T_4	Mycorrhizae	19.36	34.60
T_5	Microalgae + Neem cake	16.12	43.13
T_6	Microalgae + Vermicompost	21.80	45.33
T_7	Microalgae + Mycorrhizae	17.95	32.00
T_8	Microalgae + Neem cake + Vermicompost	22.60	50.40
T_9	Microalgae + Neem cake + Mycorrhizae	19.05	40.83
	S. Ed (±)	0.50	1.16
	C.D	1.05	2.43

The analysed data mentioned in Table 2 and Fig. 2 reveal that all the treatments considerably increased the plant height (50.40) as compared to the control. Among the applied combinations T8 – microalgae + neem cake + vermicompost significantly increased the plant height than the rest. All treatments were found to be merely similar over control. The treatments (T_6, T_5), (T_5, T_9), (T_3, T_2) and (T_2, T_7) are non-significant among themselves. Similar findings have been reported by (Emara *et al.*, 2019).

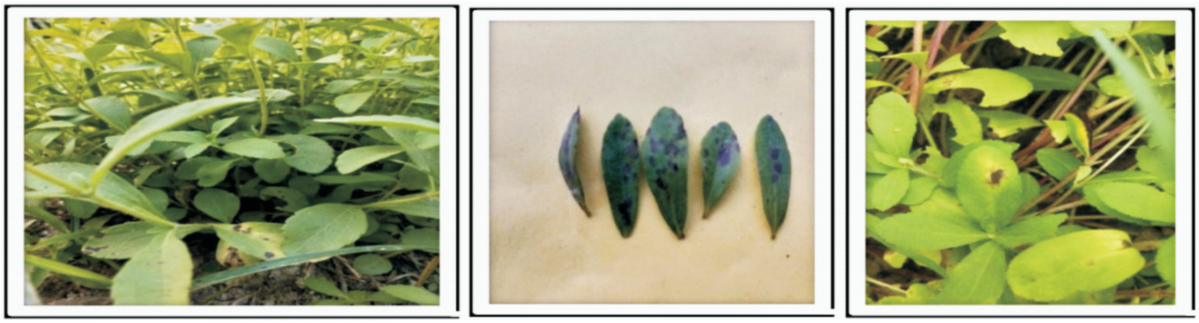

Fig. 1. Typical symptoms caused by the pathogen.

Table 2. Effect of bioresources on disease incidence of Stevia

Treatments		Disease incidence (%)		
		15 DAT	30 DAT	60 DAT
T_0	Control	32.32	42.80	49.19
T_1	Microalgae	17.03	26.79	29.05
T_2	Neem cake	23.32	28.39	38.66
T_3	Vermicompost	21.78	26.08	33.99
T_4	Mycorrhizae	25.83	29.60	39.85
T_5	Microalgae + Neem cake	11.30	21.60	26.51
T_6	Microalgae + Vermicompost	10.58	19.72	24.53
T_7	Microalgae + Mycorrhizae	24.33	28.80	39.20
T_8	Microalgae + Neem cake + Vermicompost	7.86	17.86	22.79
T_9	Microalgae + Neem cake + Mycorrhizae	14.00	25.20	27.20
	S. Ed (±)	0.72	0.70	1.08
	C.D	1.53	1.49	2.29

The statistical data represented in the Table 3 and Fig. 3 describe that number of branches significantly increased with the applied combinations in comparison to control, among the treatments T8 – microalgae + vermicompost + neem cake showed a greater number of branches (23) compared to remaining. All the treatments are merely substantial over control but, the treatments (T_8, T_6), (T_5, T_9, T_1), (T_1, T_3), (T_2, T_7) and (T_4, T_0) are observed to be not significant among them. Similar outcomes are re-

Table 3. Effect of bioresources on the number of branches of *Stevia rebaudiana*

Treatments		Number of branches	
		Before application	60 DAT
T_0	Control	14	16
T_1	Microalgae	16	21
T_2	Neem cake	15	18
T_3	Vermicompost	17	20
T_4	Mycorrhizae	15	16
T_5	Microalgae + neem cake	19	22
T_6	Microalgae + vermicompost	17	23
T_7	Microalgae + mycorrhizae	16	18
T_8	Microalgae + neem cake + vermicompost	18	24
T_9	Microalgae + neem cake + mycorrhizae	18	21
	S.Ed(±)	0.68	0.61
	C.D (5%)	1.67	1.31

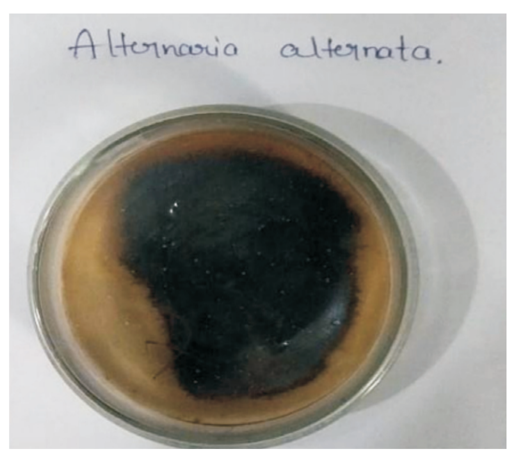

Fig. 2. Pure culture *Alternaria alternata*

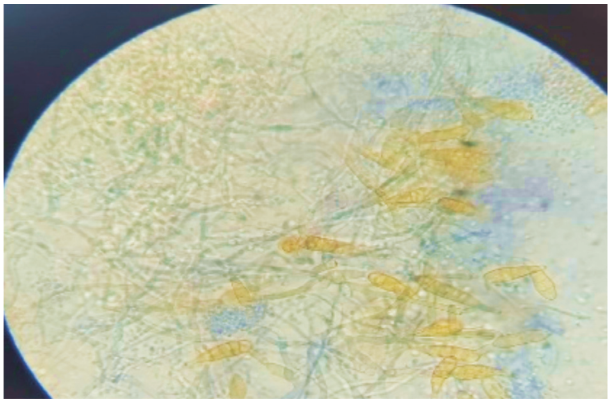

Fig. 3. Microscopic view of the plathogen

ported by (Emara *et al.,* 2019); (Maiti *et al.,* 2007).

In recent days several cutting-edge plant protective measures have emerged and are available at our fingertips, but these are ultimately resulting in heavy economic inputs and require skilled potential. But as a core aim to safeguard the crop from the deadly pathogens one has to go for these management practices. But these are prime of chemical origin which show the hazardous effect to the environment and mankind as well. Therefore, in this present experimental study eco-friendly treatment combinations were selected to develop an alternative management strategy. Inmodern era, the conventional practices in agriculture like the use of neem cake, vermicompost, microalgae and mycorrhizae resumed greater importance, it may be due to theirinhibitory potential on pathogens. From the present study, the combination of microalgae + neem cake + vermicompost was found to be the most effective against the pathogen. The reasons maybe because of their greater affinity to retard the growth of the pathogen due to the presence of chief constituent azadirachtin which possesses nearly about 100 anti-microbial agents, in addition to azadirachtin the neem leaf also contains several anti-fungal and anti-microbial agents such as Nimbin, nimibidol, gedunin, quercetin, ascorbic acid, 17-hydroxyazadiradione and many polyphenolic flavonoids. The combination of microalgae boosts up the nutrient intake of the plant which in turn supports the plant to withstand the attack of the pathogen.

Conclusion

The results revealed that the treatment combination T_8 - Microalgae + neem cake + vermicompost showed the best results and significantly increased the plant height (cm), number of branches with minimum disease incidence (%) as par with control followed by T_9 – microalgae + neem cake + mycorrhizae. To bring down the hazardous effects of chemicals on the environment and mankind, such an experimental trial has been conducted. From the present study, it is evident that these eco-friendly combinations are more effective in conquering pathogen growth and can be utilised as an alternative therapeutic practice for managing plant diseases with zero harm. However, still, there are opportunities for more research trials.

Acknowledgement

The authors are thankful to the head of the department, Department of Plant Pathology, Naini Agricultural Institute, Prayagraj, U.P., India.

References

Arab, E.A., Lopez, M.H. and Barrera-Necha, L.L. 2000. Mexico against four fungal postharvest pathogens of fruits and vegetables. *Mexical Journal of Phytopathology.* 18(1): 36-41.

Dhruva, P., Gauchan, A., Dhakal., Sharma, N., Bhandari, S., Maskey, E., Shreshta, N., Gautam, R., Giri, S and Sushma, G. 2014. Regenerative callus induction and biochemical analysis of *Stevia rebaudiana* Bertoni. *Journal of Advanced Laboratory Research in Biology.* 5: 41-45.

Dushyanth, Maji, S., Kumar, S., Maurya, A.K. and Meena, K.R. 2014. Efficacy of organic manures on growth, yield and biomolecules of stevia. *Journal of Crop and Weed.* 10(1) : 107-110.

Emara, H.A., Hamza, E.M, Hamouda, R.A., EI Masry, M.H. and EI Sayed, F.N. 2019. The efficiency of algae employment in *Stevia rebaudiana* micropropagation. *Middle East Journal of Agriculture Research.* 8 : 425-437.

Maiti, C.K., Sen, S. and Acharya, K. 2007. First report of *Alternaria alternata* causing leaf spot on *Stevia rebaudiana*. *Plant Pathology.* 56: 724.

Singh, A. and Verma, P.P.S. 2015. Survival and growth performance of stevia cutting under different growth media. *Journal of Medicinal Plant Studies.*3(2): 111-113.

Yadav, A.K., Singh, S., Dhyani, D. and Ahuja, P.S. 2010. A review on the improvement of stevia. *Canadian Journal Plant Sciences.* 91: 1-27.