Biological Forum – An International Journal **15(6): 142-147(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Encountering the effects of Leaf Spot (*Alternaria polianthi)* on Tuberose (*Polianthes tuberosa*) by Biocontrol agents, Plant extracts, and Plant Oils

P. Mahalakshmi[1*], M. Ayyandurai[2] and P. Mareeswari[2]
[1]Department of Plant Pathology,
Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[2]Department of Plant Pathology,
Agricultural College and Research Institute, Madurai (Tamil Nadu), India.

(Corresponding author: P. Mahalakshmi)*
(Received: 13 March 2023; Revised: 16 April 2023; Accepted: 25 April 2023; Published: 20 June 2023)
(Published by Research Trend)

ABSTRACT: Tuberose is an ornamental crop grown in tropical and subtropical areas. The incidence of leaf spot disease caused by the fungal pathogen *Alternaria polianthi* is a severe problem that leads to reduced growth, yield loss, and is triggered by high temperature and humidity. In response, a survey was conducted in the farmers' fields in Melachinnanampatti village, Alanganallur block, Madurai district, which recorded a maximum disease incidence of 50.34%. To manage the pathogen, bio-control agents (*Trichoderma asperellum* and *Bacillus subtilis*), plant extracts, and plant oils were tested *in vitro*. Eleven plant extracts were tested, including Neem, Ginger, Garlic, Moringa, Pungam, Calotropis, Castor, Eucalyptus, Bermuda grass, Nithya kalyani, and Bougainvillea, each with a 10% concentration. Ginger extract showed the highest inhibition (66.34%) compared to the control pathogen. Six plant oils were also tested, including Neem oil, Pungam oil, Groundnut oil, Illuppai oil, Mustard oil, and Castor oil, each with a 5% concentration. Pungam oil showed the highest inhibition (45.56%) among the plant oils. The bio-control agents *Trichoderma asperellum* and *Bacillus subtilis* displayed growth inhibition of 45.34% and 37.45%, respectively. From these results, it was concluded that biocontrol agents, plant extracts, and plant oils exhibited significant inhibition of the growth of *Alternaria polianthi*.

Keywords: *Alternaria polianthi*, Biological management, Biocontrol agents, Plant extract, plant oils.

INTRODUCTION

Tuberose (*Polianthes tuberosa*), a commercially important ornamental and medicinal plant, belongs to the family *Amaryllidaceae*. It is widely cultivated in states such as Karnataka, Gujarat, Tamil Nadu, Haryana, Punjab, Andhra Pradesh, Rajasthan, West Bengal, Assam, and Maharashtra in India (Khan and Pal 2001; Biswas *et al.*, 2002). Many economically important ornamental plants suffer from fungal diseases such as blossom blight caused by *Fusarium equiseti* and leaf spot caused by *Alternaria polianthi*. Among these, leaf spot caused by *A. polianthi* is the most threatening to this plant. In India, *A. polianthi* was first reported on tuberose (Mariappan *et al.*, 1977). *Alternaria polianthi* is a significant fungal pathogen of tuberose, causing characteristic symptoms on leaves. The symptoms consist of red-brown spots with faint concentric rings on the midrib and margins of the leaf. Dark brown spots of 10-50 mm in length appear on the peduncle. Infection leads to drying up of affected parts. The spots begin as brown specks and grow in a circular to oval form with a diameter of 4-5 mm and a length of 10-30 mm. The number of spots in each leaf vary from one to ten and spots frequently become larger and coalesce into bigger patches. Fungicides are widely used to control leaf spot disease and are usually effective, but they come with significant costs and can harm the environment, human health, and lead to resistance in fungal pathogens (Rahmatzai *et al.*, 2017). As a result, biological control of plant diseases is seen as a more viable alternative to chemical pesticides and cultural practices. Plant oils and plant extracts have antifungal properties that can control a range of plant diseases. They act by inhibiting spore germination, altering cell membrane integrity, and suppressing mycelial growth. They are also effective against pests, making them a broad-spectrum biopesticide that is safe for beneficial insects and humans. Biocontrol agents are utilized in plant disease control, including species like *Bacillus* and *Trichoderma*. By using these organisms, a more natural and sustainable approach to disease management can be achieved, reducing the dependence on synthetic pesticides. This study focuses on the role of plant oils, plant extracts and biocontrol agents in their antifungal activity against the leafspot disease of tube rose under controlled conditions.

MATERIAL AND METHODS

Survey and Collection of disease Symptoms. A field survey was conducted in Kallivelipatti, Melachinnanampatti, and Sembukudipatti villages in Alanganallur, Madurai to assess the incidence of Alternaria leaf spot disease in Tuberose. Five fields were randomly selected in Kallivelipatti, and the leaf

spot disease incidence was surveyed in Tuberose. In each field, five one square meter areas were selected. In each one square meter area, all plants were assessed for the severity of Alternaria leaf spot. The disease grades were assigned according to the standard grade chart developed by Ramakrishnan *et al.* (1971). Leaves of Tuberose exhibiting typical symptoms of leaf spot, characterized by concentric rings caused by *A. polyanthi*, were collected for isolation of pathogens.

Isolation of Pathogen. The pathogen was isolated from the diseased tissues of Tuberose using the tissue segment method (Rangaswami, 1958). The infected portions of diseased leaves were cut into small pieces with a sterilized scalpel and surface sterilized with 0.1% mercuric chloride for 1 minute, then washed three times in sterile distilled water before being placed on PDA medium in a petri dish. The plates were incubated at room temperature (28 to 32°C) for 7 days and monitored for the growth of the fungus. The hyphal tips of the growing fungus were aseptically transferred to PDA slants for maintenance of the culture. The pathogen was identified based on cultural and morphological characteristics.

Morphological Characterization of pathogen. To identify the cause of Alternaria leaf spot disease in tuberose, a morphological and cultural characterization of *A. polianthi* can be performed. The process involves the isolation of the pathogen from infected leaves. The isolated pathogen is then incubated on potato dextrose agar (PDA) medium for 7 days. During this time, the cultural characters of the isolate, such as its color, texture, margin, zonation, and length of mycelia, are observed. The morphological characteristics of the fungus, including its colony shape, texture, color, growth rate, and the presence of characteristic structures such as conidia, are also observed. Based on these observations, the pathogen can be identified.

Collection of antagonists. The antagonists *Trichoderma asperellum* and *Bacillus subtilis* were obtained from the Department of Plant Pathology at the Agricultural College and Research Institute in Madurai. The colonies were aseptically transferred to newly made PDA or nutrient agar medium. The plates were incubated at room temperature (28-32°C) for 7 days, resulting in a fresh and active culture ready for bio control experiments.

Testing of antagonists against *A. polianthi.* A dual culture assay was conducted to test the effectiveness of bio control agents *Trichoderma asperellum* and *Bacillus subtilis* against the causative organism of leaf spot in Tuberose (Dennis and Webster 1971). Fresh cultures of the agents were obtained and the fungal culture was grown on potato dextrose agar (PDA) plates for 7 days. The test fungus and bio agent were cut using a sterilized cork borer and transferred aseptically to petri plates filled with PDA. The test fungus and bio agent were placed opposite each other on the PDA, ensuring equal opportunity for growth. In the case of *Bacillus subtilis*, streaks of 24 hour old bacterial isolates were made on one side of the Petri plate and the mycelial disc of the test fungus was placed at the opposite side. The plates were incubated at room temperature for 5 days and the results were analyzed statistically to determine the effectiveness of the bio control agents against the leaf spot causing organism by using following formulae

$(1 - (A/B)) \times 100\%$

where A = growth of the target organism in the presence of the bio control agents

B = growth of the target organism in the absence of the bio control agents

Plant extract preparation. Fresh, healthy plant parts (100 g of leaves/rhizome/bulb of Neem, Ginger, Garlic, Moringa, Pungam, Calotropis, Castor, Eucalyptus, Bermuda grass, Nithya kalyani and Bougainvillea) were collected, washed with distilled water, air-dried, and crushed in 100 ml of sterile water. The crushed product was filtered using a muslin cloth. The culture filtrate was further diluted to 10%. Potato dextrose agar was used as the nutrient medium, and the required quantity of each botanical extract was added to achieve the desired concentration. The botanical extract was mixed with the PDA medium and sterilized.

Assessing efficacy of Plant extract against *A. polianthi.* About 20 ml of poisoned medium was poured to each of the 90 mm Petri dishes and allowed for solidification. The actively growing periphery of the twelve day old culture of *A. polianthi* was carefully cut using a gel cutter and transferred aseptically to the center of each Petri dish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without the plant extract. The plates were incubated at 27±1 °C for ten days and the colony diameter was recorded.

Assessing efficacy of Plant oils against *A. polianthi.* The antifungal activity of different plant oils (Neem oil, Pungam oil, Groundnut oil, Illuppai oil, Mustard oil, and Castor oil) was tested against *A. polianthi* using the poisoned food technique reported by Ahmed and Andelgaleil (2008). To prepare the medium, 0.125 ml and 0.25 ml of each plant oil was mixed with 50 ml of PDA medium to obtain 0.25% and 0.5% concentrations respectively and sterilized. 15 ml of each plant oil medium was poured into sterilized Petri plates, a 9mm mycelial disc of *A. polianthi* was placed at the center, and incubated. The control was PDA medium without plant oils. Plates were incubated for 7 days at 28±2 °C. *A. polianthi* radial growth was recorded after 7 days and the growth inhibition was calculated. The experiment was conducted using a completely randomized design with 3 replicates for each treatment.

Statistical Analysis. The experimental data was analyzed with ANOVA using SPSS 17.0. Treatment means were compared using Duncan's Multiple Range Test at a 5% significance level.

RESULTS AND DISCUSSION

Survey and Collection of disease Symptoms. A survey on the occurrence of leaf spot disease incidence in tuberose was undertaken to know the distribution and severity of leaf spot disease in tuberose growing areas of Alanganallur block, Madurai. During the survey, the severity of leaf spot disease of tuberose varied from 22.5 to 50.34 PDI. The maximum percent disease index

of 50.34 percent was recorded in Melachinnanampatti village, while it was least in Kondaiyampatti village. The survey revealed that the severity of Alternaria leaf spot in tuberose varied from location to location due to various factors like temperature, relative humidity, rainfall and cultural practices followed. Similarly, a survey was conducted in Jashore district of Bangladesh by Rabita Zanan (2019). From this survey, highest percent disease index of 65.36 was recorded in Ghokali region of Jashore district. The minimum percent disease index of 23.8 was recorded in Chandpur region of Jashore district.

Isolation of pathogens. The pathogen was isolated, purified and maintained on PDA slants for further studies. The mycelial colony of the genus *A. polianthi* produced aerial mycelia with concentric rings. The pure cultures of these isolates were maintained on PDA slants for further studies. Similarly, Mazumder *et al.* (2016) also conducted the detailed survey on incidence of leaf spot disease of tuberose and documented the *A. polianthi*.

Morphological Characterization of pathogen. In the present study, the growth characterization of the *A. polianthi* isolate on Potato Dextrose Agar medium indicated that the culture was initially grayish white in color and later turned into dark grey or black in color. The mycelium is having cottony – fluffy texture with roundish margin. No zonation is observed. The diameter of the mycelia observed on third and fourth was 8.5 to 9cm. The variation in the growth characterization of the *A. polianthi* isolates indicated that most of the cultures were gray or brown in colour with light or dark brown pigmentation and regular or irregular growth pattern (Loganathan *et al*., 2016). Zaman *et al.* (2019) observed that five isolates of *A. polianthi* exhibited significant variation in their cultural character, pigmentation and per day growth rate. The colonies of the different isolates of the pathogen varied from white to dark black, circular to irregular, smooth to rough with or without concentric zonation. Singh *et al*. (2020) reported the growth of the mycelia in the culture was grey to brown in colour, initially and then turned to dark black. The septate hyphae of the fungus were observed to be dark brown to black.

Bio-efficacy of bio control agents on linear growth of *A. polianthi*. *T. asperellum* and *B. subtilis* were tested against the tuberose Alternaria leaf spot pathogen, *A. polianthi* under *in vitro* by dual plate method. All the isolates showed significant reduction in the growth of the pathogen. The mean value from the three replication having the growth inhibition of 49.34 and 37.45 per cent over control for *T. asperellum* and *B. subtilis* respectively. Moges (2012) screened ten local antagonistic bacteria *in vivo* for suppressing the pathogen. He reported about the efficacy of five promising antagonists exhibiting higher zone of inhibition (ZOI) (38 mm and above) and percent disease control (ranging from 38.16 to 43.79%).

Ramanujam *et al.* (2015) evaluated the bacterial antagonists against leaf spot pathogen *A. polianthi* of tuberose, under *in vitro*, glasshouse and field conditions. Among the isolates tested, *P. fluorescens* (P) decreased the leaf spot incidence up to 62% and increased the flower yield up to 37 per centover control. In the present study the efficacy of *B. subtilis* was tested against *A. polianthi* under *in vitro* conditions. It showed significant reduction in the growth of the pathogen. The mean value from the three replication having the growth inhibition of 37.45 per cent over control.

Bio-efficacy of different plant extracts on the linear growth of *A. polianthi* under *in vitro* condition. Among the eleven plant extracts tested, *Zingiber officinae* (Ginger) showed the maximum inhibition of 66.34 per cent over control. This was followed by Garlic extract recorded 51.12 % over control. The least mycelial growth inhibition of 5.5 per cent over control was recorded in the castor leaf extract. Raza *et al.* (2019) reported that *Azadirachta indica* was found significantly effective in controlling the leaf spot of tuberose.

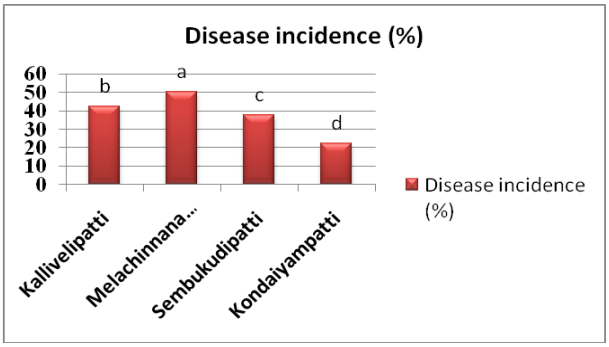

Fig. 1. Disease incidence of tuberose leaf spot in four villages.

Bio-efficacy of different plant oils on the linear growth of *A.polianthi* under *invitro* condition. Among the six plant oils tested, *Pongamia glabra* (Pungum oil) showed the maximum inhibition of 45.56 per cent over control. This was followed by Groundnut oil recorded 35.23 % over control. Whereas Mustard oil recorded least inhibition of 3.3 per cent over control. Rahmatzai (2017) tested different botanical oils on the radial growth of *A. polianthi*. The lemon oil at 3% concentration recorded the maximum growth inhibition of the pathogen by 27%. Awais *et al.* (2020) reported that contact assay method revealed that pungam essential oil was most effective to inhibit the mycelial fungal growth 89.4%, 92% and 96.2% at all applied concentrations (200, 400 and 600 ppm) followed by Moringa oil 58.9%, 65.7%, 68.3% while *Acacia karoo* showed least efficacy 46.4%, 55.3% and 59.2% respectively.

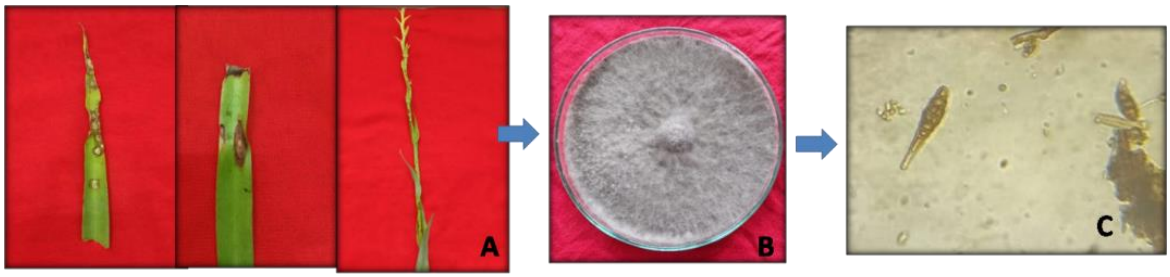

Fig. 2. Symptoms of Alternaria leaf spot disease in Tuberose; A. leaf spot of tube rose; B. Pure culture of *Alternaria polianthi*; C. Conidia of *A. polianthi.*

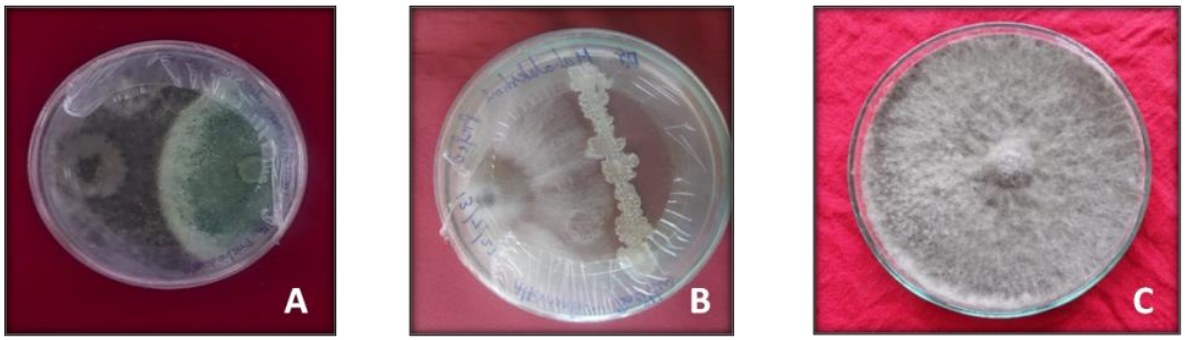

Fig. 3. Bio-efficacy of *T. asperellum* and *B. subtilis* on linear growth of *Alternaria polianthi* A. *T. asperellum* vs pathogen; B. *Bacillus* sp. vs pathogen; C. *A. polianthi.*

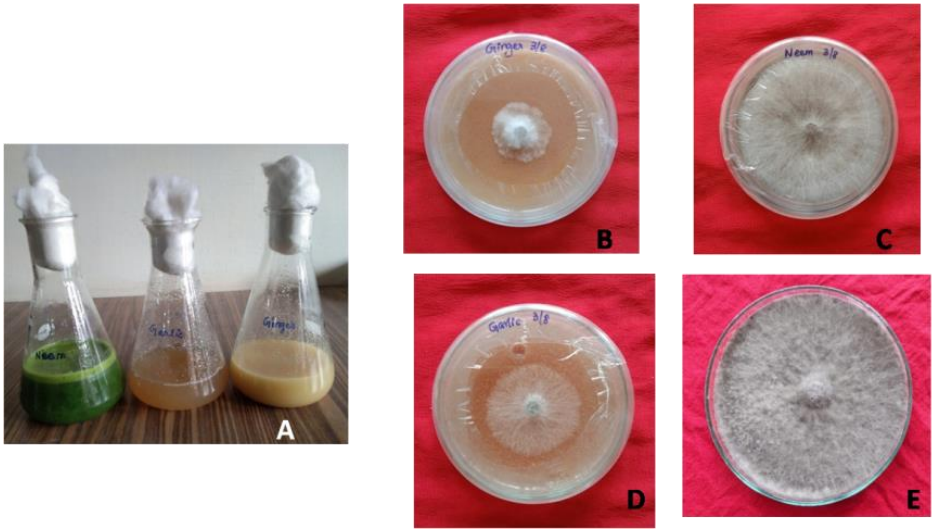

Fig. 4. Bio-efficacy of different plant extracts on the linear growth of *A. polianthi* under *in vitro* condition; A. Plant Extract; B. Ginger leaf extract; C. Neem leaf extract; D. Garlic extract; E. Control.

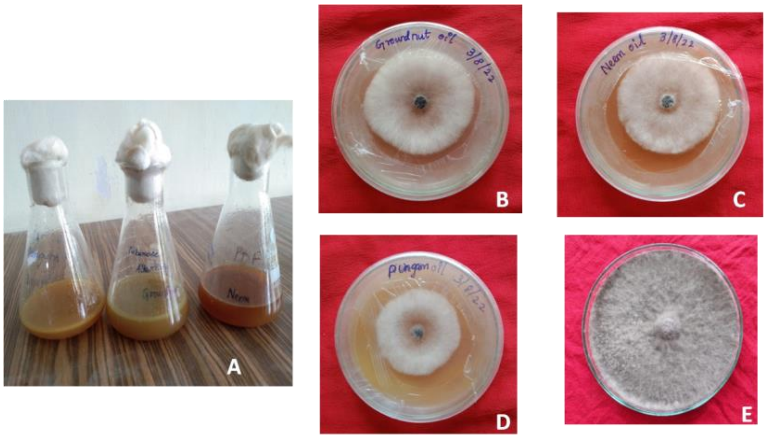

Fig. 5. Bio-efficacy of different plant oils on the linear growth of *A. polianthi* under *in vitro* condition; A. Oil extract; B. Groundnut oil; C. Neem oil; D. Pungam oil; E. Control.

Table 1: *Invitro* screening of bio control agents against *A. polianthi.*

Sr. No.	Bio control agents	Linear growth* (cm)	Percent inhibition over control (%)
1.	*T. asperellum*	4.56[c]	49.34 (44.36)[a]
2.	*B. subtilis*	5.63[b]	37.45 (37.92)[b]
3.	Control	9.0[a]	-
	CD(*P*=0.05%)	**0.25**	**1.65**

*****Means followed by the same letter differ non-significantly at P ≤ 0.05 according to DMRT; values are mean of five replications

Table 2: Effect of different plant extracts on mycelial growth of *A.polianthi.*

Sr. No.	Common Name	Scientific Name	Linear Growth* (cm)	PIOC (%)
1.	Neem	*Azadirachta indica*	7.9[c]	12.23 (20.44)[ef]
2.	Garlic	*Allium sativum*	4.4[f]	51.12 (45.63)[b]
3.	Ginger	*Zingiber officinale*	3.0[g]	66.38 (54.73)[a]
4.	Moringa	*Moringa oleifera*	4.5[f]	50.0 (44.36)[b]
5.	Pungam	*Pongamia glabra*	6.5[d]	27.7 (31.79)[d]
6.	Calotropis	*Calotropis gigantea*	7.7[c]	14.4 (22.24)[e]
7.	Castor	*Ricinus communis*	8.5[b]	5.5 (13.58)[g]
8	Eucalyptus	*Eucalyptus globules*	8.0[c]	11.11 (19.41)[f]
9.	Bermuda grass	*Cynodon dactylon*	5.3[e]	41.11 (39.86)[c]
10.	Nithya kalyani	*Catharanthus roseus*	4.6[f]	48.89 (44.36)[b]
11.	Bougainvillea	*Bougainvillea glabra*	5.1[e]	43.34 (41.16)[c]
12.	Control	-	9.0[a]	-
	CD (P=0.05%)		**0.38**	**2.67**

***** Means followed by the same letter differ non-significantly at P ≤ 0.05 according to DMRT; values are mean of three replications

Table 3: Effect of different plant oils on the linear growth of *A.polianthi.*

Sr. No.	Common Name	Scientific Name	Mycelial Growth *(cm)	PIOC (%)
1.	Neem oil	*Azadirachta indica*	6.23[c]	30.78 (33.89)[c]
2.	Pungam oil	*Pongamia glabra*	4.9[e]	45.56 (42.45)[a]
3.	Groundnut oil	*Arachis hypogea*	5.83[d]	35.23 (36.59)[b]
4.	Illupai oil	*Madhuca indica*	7.0[b]	22.22 (28.11)[d]
5.	Mustard oil	*Brassica nigra*	8.7[a]	3.33 (10.43)[e]
6.	Castor oil	*Ricinus communis*	6.53[c]	27.44 (31.79)[c]
7.	Control	-	9.00[a]	-
	CD (P=0.05%)		**0.31**	**2.46**

*****Means followed by the same letter differ non-significantly at P ≤ 0.05 according to DMRT; values are mean of three replications

CONCLUSIONS

From this study, leaf spot of tuberose is caused by *Alternaria polianthi*, which was isolated from infected symptoms and confirmed through morphological examination. The efficacy of different plant extracts, plant oil, and two biocontrol agents was assessed against the pathogen. Ginger extract, pongamia oil, and *Trichoderma asperellum* effectively inhibited the growth of the pathogen. These products will be tested under pot and field conditions to confirm their ability to manage leaf spot of tuberose.

Acknowledgement. Agricultural College and Research Institute, Department of Plant Pathology.
Conflict of Interest. None.

REFERENCES

Ahmed, S. M. and Andelgaleil, S. A. M. (2008). In vitro inhibition of plant pathogenic fungi and control of grey mould and soft root of strawberry by essential oils. *Journal of Pest Control and Environmental Sciences, Egypt*, *16*, 69-86.

Awais, S., Gulshan, I., Farah, N., Salman, G., Imran, H., Nasir, M. and Karamt, M. Z. (2020). In vitro evaluation of plant essential oils against Alternaria alternata causing fruit rot of grapes. *Asian Journal of Agriculture and Biology*, *8*(2), 168-173.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species-groups of Trichoderma: III. Hyphal interaction." *Transactions of the British Mycological Society*, *57*(3), 363-IN2.

Khan, M. R., Shit, S., Pal, A. K., & Biswas, B. (2006). Management of foliar nematode, *Aphelenchoides besseyi* infecting tuberose in West Bengal, India. *Indian Journal of Nematology*, *36*(1), 44-47.

Loganathan, M., Venkataravanappa, V., Saha, S., Rai, A. B., Tripathi, S., Rai, R. K. and Chowdappa, P. (2016). Morphological, pathogenic and molecular characterizations of Alternaria species causing early blight of tomato in Northern India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *86*, 325-330.

Mariappan, V., Babu, K. and Kandasamy, T. K. (1977). A leaf spot disease of tuberos (*Polianthes tuberosa* L.) caused by a new species of Alternaria. *Current Science*. *46*, 311.

Mazumder, N., S. K. Borah, and Preeti Hatibarua (2016). Management of leaf spot (*Alternaria polianthi*) of tuberose through fungicides." *Agricultural Science Digest-A Research Journal*, *36*(3), 250-252.

Moges, M. M., Selvaraj, T. V., & MT, J. (2012). Influence of some antagonistic bacteria against early blight

(*Alternaria solani* (Ell. & Mart.) Jones & Grout.) of tomato (*Lycopersicon Esculentum* Mill.). *The African Journal of Plant Science and Biotechnology*, *6*(1), 40-44.

Rabita Zaman (2019). Investigation and survey on Tuberose (*Polyanthes tuberose*. L) in Jashore district of Bangladesh. M.Sc. (Ag) Thesis, SHER-E-BANGLA Agricultural University, Bangladesh. pp.39-41.

Rahmatzai Najibullah (2017). Morphological and molecular characterization and management studies of (*Alternaria solani*) on". Ph.D. diss., king abdulaziz university jeddah, 2017.

Ramakrishnan, L., Kamalnathan, S. and Krishnamurthy, C.S. (1971). Studies on Alternaria leaf spot of tomato. *Madras Agricultural Journal*, *58*(4), 275–280

Ramanujam, B., Sriram, S., Rangeshwaran, R. and Basha, H. (2015). Biocontrol efficacy of fungal and bacterial antagonists against early blight of tomato caused by *Alternaria solani*. *Indian Journal of Horticulture*, *72*(1), 147-148.

Rangaswami, G. (1958). An agar block technique for isolating soil micro organisms with special reference to Pythiaceous fungi. *Sci Cult., 24*, 85.

Raza, W., Ghazanfar, M. U. and Hamid, M. I. (2019). Occurrence of late blight (*Phytophthora infestans* (Mont.) de Bary) in major potato growing areas of Punjab, Pakistan. *Sarhad Journal of Agriculture*, *35*(3), 806-815.

Singh, B., Beniwal, M., Sohi, G. S., Sran, A. S., & Kaur, P. (2020). Studies on Alternaria leaf spot of cotton with special reference to disease management by use of chemicals and botanicals. *Journal of Pharmacognosy and Phytochemistry*, *9*(5), 1857-1863.

Zaman, Rabita (2019). Investigation and survey on diseases of tuberose (*Polianthes tuberosa* L.) in Jashore district of Bangladesh". Ph.D. Thesis., Department of plant pathology, sher-e-bangla agricultural university, dhaka-1207.

How to cite this article: P. Mahalakshmi, M. Ayyandurai and P. Mareeswari (2023). Encountering the effects of Leaf Spot (*Alternaria polianthi)* on Tuberose (*Polianthes tuberosa*) by Biocontrol agents, Plant extracts, and Plant Oils. *Biological Forum – An International Journal, 15*(6): 142-147.