Suppressive capability of fungicides and botanical extracts against *Alternaria polianthi* Causing leaf spot of *Tuberose (polianthus tuberosa L)*.

MAHENDRA BASAPPA WAGHMARE

Department of Botany The New College, Kolhapur 416012, India.

ABSTRACT

Alternaria species causing diseases to horticultural and medicinally important ornamental plants. In the present study the experiment was made on the leaf spot disease of tuberose *(polianthus tuberosa).* This fungus produces profuse dark brown to blackish mycelium with transverse as well as longitudinal septate conidia on the czapex dox agar medium. In this study attempts was made to investigate the constraint capability of some fungicides and plant extracts against *Alternaria polianthi* causing leaf spot of tuberose. In *vitro* study fungicides, Viz. mancozeb, carbendazim, coper oxychloride,chlorothalonil and propiconazole and Leaf extracts of *Saraca indica* Linn. *Cassia auriculata* L. *pongamia pinnata* L *Vitex negundo* L., *Boerhavia repens Var. diffusa* (L.). Hook. *Tithonia diverisfolia* A.Gary used against the pathogen by the Poisoned food technique. Among the tested fungicides mancozeb 1000 ppm was found most effective against the pathogen it shown 90.74% Percent of inhibition, followed by carbendazim 87.41%, copper oxychloride 84.07% and cholorothalonil shown 81.85% and propicanozole shown 80.74 % of growth inhibition . Among the plant extracts 50 % alcoholic leaf extract of all tested plants inhibited the growth of the pathogen. 100 % aqueous leaf extract of *Saraca indica* was quite superior over the other tested plant extracts, it shown maximum 86.33% percent of growth inhibition .

Key words: *Alternaria polianthi,* Fungicides, Plant extracts. *Polianthus tuberosa* ,

Polianthus tuberosa, commonly known as gulchaddi or nishigandha or rajanigandha. It is an important ornamental and medicinal perennial herb, belongs to the family Amaryllidaceae. It is night blooming bulbous plant reaching up to 2 to3 feet height. It is widely grown in tropical and subtropical areas (Bahadoran *et al.*2015). It is commercially cultivated in many countries of the world like India, Hawaii, China, Brazil, Italy, Iran, UK, USA etc. In Maharashtra, because of its scented flowers it is mostly used in the perfume industry (Mishra *et al.* 2008). In Ayurveda bulbs are used as anti-gonorrhea, diuretic, emetic , curing rashes and to remove the small red pimples form the new borne childs. Oil obtained from the scented flowers is very expensive. In aromatherapy, it is used in management of anxiety, stress, anger, confusion, emotional disturbances, and insomnia. The essential oil is found to be active against gram-positive and gram-negative bacteria. Flowers also used to making the garlands, bouquets decoration and wreath (Amin *et al.* 2017). Flowers contain glycosides, spirostanols, polianthosides and furostanols (Roshani *et al.* 2019). Three glycosides has been isolated from the bulbs of the plant (Kha *et al.*2002). Such medicinally and commercially important ornamental plant suffering from the several fungal diseases which reduced the economic value and yield of the plant . This is susceptible to many diseases caused by fungi, bacteria and nematodes. Among fungal diseases, stem rot or tuber rot caused by *Sclerotium rolfsii* Sacc. flower blight caused by *Botrytis cinerea,* Blossom blight caused by *Fusarium equisetti* and leaf spot caused by *Alternaria polianthi* . Among all the fungal disease leaf spot disease caused by *ALternaria polianthi* was very threatened to the plant (Mariappan *et al.*1977). Symptoms observed on the leaf. *Alternaria* species are mainly saprophytic and usually found on the decaying plant debris or in the soil. In the current work attempt were made to evaluate the efficacy of fungicides and some plant extracts against the *Alternaria polianthi.* This research work will definitely helpful to the cultivars and the workers who are in the field of fungicide resistance and eco friendly disease management.

MATERIALS AND METHODS

***Experimental site and sample collection*:** The study was carried out during the 2017-2018 at the Department of Applied Botany, The New College, Kolhapur, Maharashtra (India). The Experiment site located at 16 ^{0} 41' N and 74 ^{0} 14' E at an elevation about 546 M. The samples of naturally infected leaf and stem were collected from the Kolhapur district (Rashiwade and Shirol) for the said study.

Isolation and morphological study of the pathogen: Naturally infected leaf and flowers of tuberose which exhibiting the typical symptoms of leaf spots were surveyed and collected in the sterilized glass bottles and brought to the laboratory. In the Laboratory washed the collected sample with sterile distilled water and made the small pieces with sterile blade, Sterilized the small cut pieces with sodium hypo chloride solution of 5% concentration for 5 minutes. Again 2 times washed the infected samples with sterile distilled water and removed the water traces from the plant material with the help of blotting paper. Later on transferred the infected tissues on the sterile solidified CDA plates and kept on incubation at 25-30 ^{0}C in the BOD incubator. The fungal mycelial growth was observed after 8 days of the incubation. Identified the pathogen as *Alternaria polianthi* followed by (Barnett and Hunter, 1972; Subramanian, 1971). Obtained the pure culture and maintain in the BOD incubator at 5^{0}C for further study. The pathogen was confirmed by the Koch postulates. The Morphological and *in vitro* suppressive capacity of the fungicides and plant extracts was carried from the 8 days old and active culture of the fungus.

***Preparation of plant extract*:** Fresh and healthy leaves of *Saraca Indica* Linn. *Cassia auriculata* L. *pongamia pinnata* L *Vitex negundo* L., *Boeraavia repens Var. diffusa* (L.). Hook. *Tithonia diverisfolia* A.Gary were collected from Shivaji University campus and surrounding area of The New College kolhapur. Brought the fresh material to the botany laboratory and washed thoroughly and oven dried, the dried leaves were pulverized to powder. Leaf extracts of each plant species were prepared with 95% ethanol (1:5 w/v) in a beaker and boiled in hot water bath for 30 minutes. The material was homogenized and filtered through double layered muslin cloth the collected extract was considered as 100% concentration of stock solution this stored in conical flask for further study. As per the requirement the stock solution of the plant extracts diluted at 25, 50, 75 and 100 % with sterile distilled water . Similar process followed for the extraction of aqueous plant extracts.

In Vitro evaluation of plant extracts: The experiment was carried for the determination of percent of growth inhibition by measuring the Mycelial growth following the food poisoning technique (Mishra and Tiwari,1992) at four different concentrations. The standardized concentration of alcoholic and aqueous plant extracts were amended in the sterilized CDA medium and this amended medium poured in the sterilized Petri plates separately. Test fungus was multiplied on Petri plates containing CDA, supplemented with alcoholic plant extracts at four concentrations 25, 50,75 and 100 % . Plates Inoculated with 8 mm disc of fungal culture taken from actively growing 7 days old culture mycelium of *Alternaria polianthi* and the plates were kept upside down, each treatment was replicated thrice. The plates were incubated in BOD incubator at 25°C, plates without plant extracts was served as control. The radial growth of mycelium measured after 8 days of the inoculation. Similar experiment was undertaken for aqueous extracts and determined the percent growth inhibition followed by (Vincent, 1947)

In Vitro evaluation of Fungicides:

The fungicides mancozeb, carbendazim, coper oxychloride, chlorothalonil and propiconazole were tested *in vitro* against *Alternaria polianthi* by poisoned food technique Different concentration of fungicides viz 200,400,600,800, and 1000 ppm of each fungicides incorporated separately in to 100 ml of sterilized CDA medium mixed uniformly and poured in to sterilized Petri plates. 8 mm discs of actively growing 8 days old culture was inoculated on the medium and after 8 days measured the radial growth of the pathogen and determined the percent inhibition of the mycelium growth over the control. Plates without any fungicides treated as control.

The percent inhibition of the mycelium growth over the control was calculated by (Vincent, 1947)

$$PI = \frac{C-T}{C} \times 100$$

Where PI= Percent Inhibition

C = Control (Mycelial growth of the fungus in the absent of treatment)

T- Treated (Mycelial growth of the fungus with treatment).

RESULT AND DISCUSSION

Symptoms and Morphology of the pathogen *:* The symptoms of leaf spot disease firstly found as small black or dark brown spots on the upper surface of the leaves and on the stem. These spots extend and covered the whole surface of the leaf lamina. In the severe condition symptoms also observed on the flowers. Ito *et al.* (2004) reported that the *Alternaria* species produces the host selective toxins. The pathogen can easily identified on the basis of morphology of the conidia and pattern of the conidia formation. The conidia formed separately or in the chain, ovoid to obovate, pale brown to dark brown, multi celled or muriform and beak is mostly present (Meena *et al.* 2010) .

In Vitro efficacy of Fungicides*:* Fungicides tested *in vitro* against *Alternaria polianthi* variably reduced the growth of the fungus (Table1). Among the tested fungicides 1000 ppm of mancozeb was more effective in the mycelial growth inhibition of *Alternaria polianthi* (90.74 %). followed by carbendazim (87.41%) , copper oxychoride (84.07%) and chlorothalonil (81.85%) and propiconazole shown 80.74 % inhibition. These results are in agreement with previous workers, According to Krishna *et al.* (1998) mancozeb was more superior than the copper-oxychloride and carbendazim against the *Alternaria* sp. Ghosh *et al*. (2002) reported that mancozeb was most effective against mycelial growth of *Alternaria alternata* causing leaf spot of gerbera. Hossain and Mian, (2004) shown that Mancozeb, Carbendazim and Propiconazole reduced the mycelial growth of the *Alternaria brassicicola* infecting cabbage. Lalesh kumara, (2006) found that mancozeb significantly inhibited the mycelial growth of *A. alternata* causing leaf spot of *chrysanthemum.* Arunkumar (2008) reported that carbendazim and thiophanate methyl at various concentrations reduced the growth of *Alternaria alternata* caused leaf blight of *chrysanthemum.* Meena *et al.*(2010) shown a least disease intensity in the mancozeb foliar spray against *Alternaria* blight disease of Indian mustard. Kantawa *et al.* (2014) stated that mancozeb at 1000 ppm inhibited the growth of *Alternaria alternata* causing leaf spot of groundnut. TejaKumar and Devappa (2016) shown that mancozeb at 1000 and and difenconazole at 2000 ppm inhibited the growth of *Alternaria alternata* and *Cercospora capsici* infecting leaf spot of chilli. The mixture of carbendazim and Mancozeb was more efficient against the *Alternaria alternata* causing leaf and fruit spot in pomegranate (Vasudha *et al.*2018). Rajput and Choudhari, (2018) observed that mancozeb and carbendazim were most effective against *Alternria alternata* causing leaf spot of brinjal. Valvi *et al.*(2019) recorded the minimum disease incidence with Mancozeb against the *Alternaria brasicae* causing leaf spot of cauliflower.

Table 1. *InVitro* efficiency of fungicides against mycelial growth of *Alternaria polianthi* casuing leaf spot of Tuberose**.**

Name of fungicides		Percent inhibition of mycelial growth at ppm					
Technical name	Trade name	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	Average %
Mancozeb	Dithane (M -45)	17.41	34.07	52.96	87.41	90.74.	56.51
Carbendazim	Bavistin (50 WP)	15.18	31.85	49.33	85.18	87.41	53.79
Copper oxychoride	Blitox (50 WP)	12.96	28.52	47.41	82.96	84.07	51.18
Chlorothalonil	Kavach (75 WP)	11.85	25.85	44.07	79.63	81.85	48.65
Propiconazole	Tilt (25% EC)	09.63	22.96	41.85	76.33	80.74	46.30
control	----------	00.00	00.00	00.00	00.00	00.00	00.00
SEm±		**1.40**	**1.8**	**1.7**	**1.7**	**1.64**	

In Vitro efficacy of plant extracts : efficacy of five plant extracts each at four concentration (25, 50,75 and 100 %) was tested against *Alternaria polianthi* causing leaf spot of Tuberose. Plant extracts are environmental friendly hence it is widely used in the field of disease and pest management of medicinal and aromatic plants. In the present research work it is found that all 50 % alcoholic leaf extracts of tested plants inhibited the growth o the pathogen while 100 % aqueous leaf extract of *Saraca indica Linn* shown 86.33% , *Cassia auriculata L.* 81.18 % , *pongamia pinnata* L., 81.85% ,*Vitex negundo* L., 79.63%, *Boerhavia repens Var. diffusa* (L.). Hook 76.33% and *Tithonia diverisfolia* A.Gary, showed 72.96 % of growth inhibition against the pathogen. (Table 2). These findings are in comfortable with the previous workers. Shivpuri *et al.* (1997) found ethanol plant extract were more toxic to *Alternaria brassicola.* Krishna *et al.* (2001) reported 25% concentration aqueous and ethanol leaf extracts of *Datura metel, Lawsonia inermis* and *Sphaeranthus indicus* completely inhibited the conidial germination of *Cercospora personata* causing leaf spot of groundnut . Alcoholic leaf extracts of *Tridax procumbens, Lantana camara, Ocimum sanctum* shown 100% efficacy against *Alternaria spinaceae* (Bhale *et al.* 2009). Waghmare *et al.* (2010), stated that alcoholic leaf extracts of *Melia azedarach L. Clerodendrum inerme L,* and *Tagetes erecta L.* inhibited growth of *Alternaria alternata* causing leaf blight of rose.

Table2. *In Vitro* efficiency of Alcoholic and aqueous plant extracts against mycelial growth of *Alternaria polianthi* casuing leaf spot of Tuberose

Name of the Plant	Percent inhibition of mycelial growth at Percent							
	aqueous plant extracts in %				alcoholic plant extracts in %			
	25%	50%	75%	100%	25%	50%	75%	100%
Saraca Indica Linn.	50.74	60.74	71.85	86.33	82.96	00.00	00.00	00.00
Cassia auriculata L.	48.52	59.63	69.63	85.18	81.85	00.00	00.00	00.00
pongamia pinnata L	47.41	57.41	67.41	81.85	79.63	00.00	00.00	00.00
Vitex negundo L.,	45.18	54.07	65.18	79.63	77.41	00.00	00.00	00.00
Boeraavia repens Var. diffusa (L.). Hook	42.96	51.85	62.96	76.33	75.11	00.00	00.00	00.00
Tithonia diverisfolia A.Gary	39.63	49.63	59.63	72.96	72.96	00.00	00.00	00.00
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
S.Em ±	1.50	1.65	1.63	1.92	1.35	00	00	00

REFRENCES

Amin M R, Pervin N, Nusrat A, Mehraj H, Jamal Uddin AFM. 2017. Effect of plant growth regulators on growth and flowering of Tuberose (*Polianthes tuberosa* L.) cv. single. *Journal of Bioscience and Agriculture Research* 12(1), 1016-1020.

Arunkumar G S. 2008. Studies on leaf blight of chrysanthemum caused by *Alternaria alternata* (Fr.) Keissler. M.Sc. (Agri) Thesis, TNAU, Coimbatore .

Bahadoran M, Salehi H. 2015. Growth and flowering of two Tuberose (*Polianthes tuberosa* L.) cultivars under deficit irrigation by saline water. *Journal of Agricultural Science and Technology* 17, 415-426.

Barnett H L and Hunter B B.1972. Illustrated Genera of Imperfect Fungi., *APS press, Minnesota*, USA. 218

Bhale U N, Rajkonda J N. and Sarwade P P. 2009. Evaluation of botanicals against *Alternaria spinaceae* causing leaf spot of spinach (*Spinacea oleracea L.),Geobios,* 36:125-128.

Ghosh C, Pawar N B, Kshirsagar C R. and Jadhav A C. 2002. Studies on management of leaf

spot caused by *Alternaria alternata* on gerbera. *J. Maharastra Agril. Univ***. 27:** 165-167

Hossain M S and Mian IH. 2005. Effect of foliar fungicides on the control of Alternaria blight of Cabbage seed crop. *Bangladesh J Pl. Pathol.* 20:43-48.

Ito K, Tanaka A, Hatta R, Yamamoto M, Akimistu K and Tsuge T.2004. Dissection of the host range of the fungal plant pathogen *Alternaria alternata* by modification of secondary metabolism. *Molecular microbiology* **52:** 399-411.

Kantwa S L, Tetarwal J P, and Shekhawat KS. 2014. In vitro effect of fungicides and phyto-extracts against *Alternaria alternata* causing leaf blight of groundnut IOSR *Journal of Agriculture and Veterinary Science* (IOSR-JAVS) 7(6) : 28-31

Kha KM, Perveen S, Ayatollahi SA, Saba N, Rashid A, Firdous S. 2002. Isolation and structure elucidation of three glycosides and a long chain alcohol from *Polianthes tuberosa* Linn. *Nat Prod Lett.*

Krishna K, Akbar A F M, Sastry R K, Reddy TV and Gour T B. 1998. In vitro evaluation of fungicides against *Alternaria carthami* Chowdury incitant of leaf spot of safflower. *Indian J. Pl. Protec.* 26 (2) : 181-182.
Krishna Kishore, Pande S and J Narayana Rao. 2001. Control of Late Leaf Spot of Groundnut (Arachis hypogaea L.) by Extracts from Non-Host Plant Species *Plant Pathol. J.* **17(5):** 264-270.

Lalesh kumari, Shekhawat KS, Rai PK. 2006. Efficacy of fungicides and plant extracts against *Alternaria* blight of periwrinkle (*Catharanthu roseus*). *J Mycol. Pl. Pathol*. 36(2):134-137.

Mariappan V, Babu K. and Kandasamy T K. 1977. Leaf spot disease of tuberose (*Polianthes tuberosa* L.) caused by new species of *Alternaria. Curr. Sci.* 46: 311

Meena P D, Chattopadhyay C, Kumar A, Awasthi RP, SinghR, Kaur S, Thomas L, Goyal P and Chand P.2010. Comparative study of the effect of chemicals on *Alternaria* blight in Indian mustard multi location study in India.Journal of Environmental Biology 32(3): 375-89.

Meena PD, Awasthi Rp, Chattopadhyay C, Kolte S J and Kumar A. 2010 Alternaria blight: a Chronic disease in rapeseed- mustard. *Journal of Oilseed Brassica* 1(1): 1-11.

Mishra A,Tiwary AK and Sharma J P. 2008. Effect of bio-control agent and chemicals on root rot and yield attributes in tuberose (*Polianthes tubarosa* Linn.) cultivars Calcutta Double. *J. Ornamental Hort.* 11: 41-44

Mishra and Tiwari. 1992. Toxicity of *Polyalthia longofolia* against fungal pathogens of rice. *Indian Phytopathology*. **45(1):**192-198.

Rajput R B and Chaudhari SR.2018. Screening of fungicides against *Alternaria alternata* (Fr.) Keissler In vitro condition *International Journal of Chemical Studies* 6(4): 971-974

Roshni Nahar Rahmatullah, Khoshnur Jannat, Maidul Islam, Taufiq Rahman, Rownak Jahan and Mohammed Rahmatullah. 2019. A short review of polyanthus tuberose considered as medicinal plant in bangaladesh. *Journal of medicinal plant studies.* (7)1: 01-03

Shivpuri A, Sharma O P. and Jhamaria S L. 1997. Fungi toxic properties of plant extracts against pathogenic fungi. J. *Mycol. Pl.Pathol*.27:29

Subramanian, CV.1971. Hyphomycetes. An account of Indian species except Cercospora. *Indian Council of Agric. Research*, New Delhi, pp.930.

ThejakumarM B.and DevappaV.2016.Efficacy of different fungicides against *A.alternata* and *Cercospora capsici* under in-vitro condition *Int. J. Adv. Res. Biol. Sci*. 3(5): 126-129.

Valvi HT, Saykar AD , and Bangar VR. 2019. In vitro and in vivo field efficacy of different fungicides against *Alternaria brassicae* (Berk.) *Journal of Pharmacognosy and Phytochemistry* 8(2): 1333-1337.

Vasudha A, Kadam D N, Dhutraj and Pawar D V.2018 In vitro evaluation of Different Fungicides against *Alternaria alternata* Causing Leaf and Fruit Spot in Pomegranate *Int.J.Curr.Microbiol.App.Sci* **7(10):** 2292-2298

Vincent J M. 1947. The ester of 4 hydrxy benzadoic and related compound, I method for the study of their fungi static properties *J.Soc.Chem. Ind. Landon*. 16: 746-755.

Waghmare M B, Waghmare R.M. and Kamble S S. 2010. Effect of different plant extracts on the growth of *Alternaria alternata* causing leaf spot of rose. *Bioinfolet* 7(3): 239-240.