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Study on isolation, purification and identification of gladiolus disease

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Abstract
Flower plays an important role in people's celebration and everyday lives. Among the floriculture, gladiolus is one of them. Cultivation of gladiolus flower produces huge emphasis due to less maintenance and high economic return. Even though, the diseases on gladiolus have major economic impact on quality and quantity. The objective of study was to record fungal disease (s) on gladiolus. The gladiolus crop was found infected with different fungal diseases *i.e.* wilt (*Fusarium oxysporum* f. sp. *gladioli*), corm rot (*Rhizoctonia solani*) and leaf spot (*Alternaria alternata*) during survey in *kharif* 2012. Therefore, by considering the economic losses, an investigations and experiments were carried out during 2012-2013 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra). The observations on Pathogenicity of *Fusarium oxysporum* f. sp. *gladioli*, *Rhizoctonia solani* and *Alternaria alternata* which caused wilt, corms rot and leaf spot disease on gladiolus, respectively was proved. The *Curvularia lunata* was invariably isolated from diseased samples but showed non-pathogenic reaction on gladiolus.

Keywords: fungal diseases, isolation, identification, pathogenicity and PDI

Introduction
Gladiolus is a tender herbaceous perennial. It occupies fourth of worlds bulbous flower plant area (Bose *et al.*, 2003) [4]. In India, it ranks second in area and production. World would not have been as beautiful, charming and cherishing as it is today, without flowers. Bulbous flowering plants are one of the most wonderful creations of nature. The various bulbous flowering plants provide glamour, perfection and colour. Gladiolus (*Gladiolus grandiflorus* Hort.) easily tops the list and can rightly be called the "Queen of bulbous flower crops" grown in many parts of the world (Kaikal and Nauriyal, 1964) [9].

Gladiolus plants have national and international value in respect to cut flowers. Cut flower cultivation is a sub division of ornamental plant production having the largest part in either production or economic value. The reason for the higher cultivation percentage of gladiolus is that, this species is grown for exportation. Gladiolus has a considerably high marketing rate with the production of bulbs (corms, cormels), in addition to cut flowers. On international level, gladiolus corms are used in food and in ethno medicines.

It is also called as 'Sword lily' on account of the shape of its leaves (Randhawa and Mukhopadhyay, 1986). In Europe it is commonly called as 'Corn flag' due to its infestation as a weed (Bose and Yadaw, 1989) [5].

In India, gladiolus was grown more than 1270 ha with an annual production of 150 million spikes (Arora, 2002) [1]. In the last two decades, it has become very popular flowering plant in India. The major area being Kalimpong (West Bengal), New Delhi, Srinagar (Jammu and Kashmir), Nainital (Uttaranchal), Pune and Nasik (Maharashtra), Bangalore (Karnataka), Hyderabad (Andhra Pradesh) and its cultivation is rapidly expanding in the states like Andhra Pradesh, Haryana, Karnataka, Kerala, Maharashtra, Punjab, Uttar Pradesh, Uttaranchal, Tamil Nadu and West Bengal (Naveen and Raju, 2007) [13].

Gladiolus plants have national and international value in respect to cut flowers. Cut flower cultivation is a sub division of ornamental plant production having the largest part in either production or economic value.

The gladiolus crop was found infected with different fungal diseases *i.e.* wilt (*Fusarium oxysporum* f. sp. *gladioli*), corm rot (*Rhizoctonia solani*) and leaf spot (*Alternaria alternata*) during survey.

Although, the fungal diseases had became very severe nowadays not much work is carried out on fungal diseases of gladiolus. In view of this, there is a need for systematic work which includes survey Isolation and identification for knowing disease incidence. This will enable us to know the occurrence of fungal diseases under Akola condition. In addition, it will help us to know their characteristic symptoms. It gives an idea about development of disease and to find out resistant source for the management of the disease.

Material and Methods

The present investigation was carried out during 2012-2013 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) to know the Fungal diseases of gladiolus cause severe reduction in yield and qualitative losses of flower. The present investigation deals with occurrence of different fungal diseases on gladiolus.

The glassware *viz*., Petri plates, test tubes, conical flasks of 250 ml, 500 ml and 1000 ml, funnel, beakers, pipettes, measuring cylinder, slides, cover slips, glass rods were used and Standard laboratory equipments *viz*., autoclave, incubator, laminar air flow, research microscope, stereoscopic binocular microscope, refrigerator, hot air oven, digital weighting balance, Bunsen burner were used and others materials *viz*., Blotter paper, non-adsorbent cotton, muslin cloth, cork borer, inoculating needle, forceps, potato, dextrose, agar-agar, sterilized soil, pots etc. these are the following equipments were used during the course of research work.

Methods

Survey: A survey of commercially cultivated gladiolus fields in the villages i.e. Patur, Washimba from Akola district of Maharashtra and Horticulture section field, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola was conducted during June to December 2012 for the occurrence and distribution of fungal diseases on gladiolus. Plant parts showing typical disease symptoms were collected in separate bag and were brought to the laboratory. All samples collected from different locations were subjected to isolation on PDA in the laboratory.

Collection of diseased samples

Infected corms, roots and leaves of gladiolus were collected from Horticulture section field, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and farmers' fields around Akola and examined in the laboratory for external symptoms and preserved for isolation of disease causing pathogens.

Sterilization of glass wares

For laboratory studies, standard pure chemicals and glass wares such as petri plates, slides, cover slips, beakers, conical flasks, test tubes etc. were used. Glass wares were cleaned by washing with detergent, dried and sterilized in hot air oven at 180^0C for one hour before use. Soil sterilization was done with 1% formalin. Distilled water and media were sterilized in an autoclave at 1.04 kg/cm^2 for 15 minutes.

Preparation of culture medium (PDA)

Healthy peeled potatoes 200 g were cut into pieces and boiled in 500 ml sterilized distilled water in souce pan for 30 minutes. Extract was strained through muslin cloth and quantity was measured. In remaining 500 ml water, 20 g agar-agar and 20 g dextrose were dissolved by heating. Both were mixed and volume was made up to 1 L. The medium was filtered through muslin cloth and poured into conical flasks and test tubes, then plugged with non-absorbent cotton and autoclaved at 1.04 kg/cm^2 for 15 minutes. Autoclaved tubes were kept in slanting position to obtain slants for maintenance of cultures.

Isolation of pathogens

Isolation of fungal pathogens was done on potato dextrose agar (PDA) medium. Approximately 20 ml autoclaved PDA was poured in each sterilized Petri dish and allowed to solidify. The collected diseased sample parts were cleaned properly. The diseased portion was cut into pieces (2 mm) along with healthy portion with sterile blade and transferred into sterile Petri plate containing 0.1% mercuric chloride ($HgCl_2$) solution for surface sterilization. Then pieces were transferred to sterile water and washed with 3 changes of sterilized water to remove the traces of mercuric chloride. Pieces were then dried on sterilized filter paper to remove the excess water. Four pieces of each was aseptically transferred on to solidified PDA medium in sterile Petri dish at equal distance and kept for incubation at room temperature (28 ± 2^0C). All the operations were carried out in aseptic condition. Growth of organism was observed regularly. The fungus observed around the infected bits was transferred on plates. The slides were prepared and examined under research microscope for identification.

Purification and maintenance of culture

Fungal culture was purified by hyphal tip method and transferred on PDA slants for further use.

Identification of the pathogens

The isolates of *Fusarium oxysporum* f. sp. *gladioli*, *Rhizoctonia solani*, *Alternaria alternata*, *Curvularia lunata* were identified on the basis of their morphological characteristics, microscopic structures and types of spores produced and pigmentation by using identification keys of Barnet and Hunter (1972) [2] and Barron (1968) [3].

Methodology

Preparation of mass culture: Culture of *Fusarium oxysporum* f. sp. *gladioli* and *Rhizoctonia solani* were grown separately on sand grain meal medium from seven days culture growth on PDA in petri plates. Sand-grain meal medium was prepared in the proportion 95:5 in order to get maximum inoculums of the fungus. About 400 g of sand-grain meal medium was taken in 1000 ml flasks and watered to 20 per cent of its weight and sterilized at 1.33 kg/sq.cm for one hour. Pure culture of *Fusarium oxysporum* f. sp. *gladioli and Rhizoctonia solani* were inoculated separately to the flask under aseptic condition and incubated at 27±1ºC for 15 days. The flasks were shaken on alternate days to get uniform growth. The mass culture so obtained was used for preparing sick soil for further studies.

Pathogenicity

For proving pathogenicity *Fusarium oxysporum* f. sp. *gladioli* and *Rhizoctonia solani* were tested. 100 g an inoculums of the pathogen were incorporated separately in the pot containing sterilized soil. The inoculums of pathogen were multiplied on sorghum grains. The planting materials were sown and the initiations of symptom were noted.

For proving the pathogenicity of *Alternaria alternata* the healthy one month old plants were selected, injuries were made on the leaves with carborendom powder. The seven

days old culture was inoculated and the observations were recorded for the development of disease symptoms.
The infected leaves/ corms/ roots were used for re-isolation as per procedure. The cultures obtained were confirmed for growth with original from which the isolations were made.

Result and Discussion

Pathogenicity

The four isolated fungal organisms *viz.*, *Fusarium oxysporum* f. sp. *gladioli*, *Rhizoctonia solani*, *Alternaria alternata* and *Curvularia lunata* were tested for their ability to cause disease on gladiolus. The pathogenic reaction of the fungal isolates is depicted in Table 1.

Table 1: Pathogenic reaction of the isolated fungi on gladiolus plants

Sr. No.	Isolated pathogenic fungi	Pathogenic reaction
1	*Fusarium oxysporum* f. sp. *gladioli*	+
2	*Rhizoctonia solani*	+
3	*Alternaria alternata*	+
4	*Curvularia lunata*	-

'+' – Disease symptoms,
'-' – No symptoms.

Among the four fungal isolates, *Fusarium oxysporum* f. sp. *gladioli*, *Rhizoctonia solani* and *Alternaria alternata* were found pathogenic to on gladiolus plants, while *Curvularia lunata* showed non-pathogenic reaction to gladiolus. The reisolation of the pathogens i.e. *Fusarium oxysporum* f. sp. *gladioli*, *Rhizoctonia solani* and *Alternaria alternata* from artificially infected plants proved their pathogenicity on gladiolus plant.

Fusarium wilt (*Fusarium oxysporum* f. sp. *gladioli*)

The isolation of the pathogen was made and the fungus was identified as *Fusarium oxysporum* f. sp. *gladioli.* The fungus produced micro and macro conidia. Micro conidia were globose and oval in shape, macro conidia were curved and dominantly three septate.
The initial symptoms of Fusarium wilt on gladiolus plant were yellowing of lower leaves, which started from the tip downwards. Yellowing of leaves were started 35 days after inoculation (DAI), later it progressed and complete plant showed yellowing symptoms. Yellowing ultimately lead to necrosis and death of the plant. Complete wilting of plants was observed 45 DAI (Plate 1).
McCulloch (1944) observed symptoms on leaves, corms and roots of gladiolus with a uniform infection on the whole vascular system, where yellowing and drying of the leaves was seen. In corms, the symptoms varied from a slight discoloration at the base which lead to complete rot. In case of roots, disease was first indicated with rusty colour which later became dirty and blackened.
Singh (1969) [16]. observed same types of symptoms on gladiolus plants when it was planted in National botanical garden, Lukhnow and infected with wilt disease. The causal agent was identified as *Fusarium oxysporum* f. sp. *gladioli*.
Explained the characteristic symptoms of the wilt disease on gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli*. The symptoms included inter-venial leaf tip yellowing which extended down the leaf and whole leaf gradually turned brown and became narrow. As infection advanced, the plant suddenly wilted or turned yellow and premature death of the plant was observed. The centre of the bulb turned black and rotted completely. Corms were depressed, leading to mummified corms.

Fig 1: Wilt disease on gladiolus caused by *Fusarium oxysporum* f.sp. *gladioli*.

Similar type of symptoms on gladiolus were also reported by Fulsunder *et al.* (2009) [7]. Who observed yellowing and wilting symptoms on gladiolus and micro and macro conidia in pure culture of causal pathogen *Fusarium oxysporum* f. sp. *gladioli.*

Corms rot (*Rhizoctonia solani*)

Gladiolus was found infected with corm rot disease. Initial symptoms observed on seedling as water soaked lesions at collar region which latter turned into brown to dark brown necrotic area followed by collapse of seedling. Discoloration of corms and roots was noticed and sudden death of plant was observed. Numerous blackish sclerotia were observed on dead stem and leaves. In advanced stage complete rotting of corms and roots were observed.

The fungus associated with corm and root rot was identified as *Rhizoctonia solani* on the basis of morphological characters i.e. small and smooth sclerotia, light coloured hypha, acute branching in mycelium and presence of septa at branching in hypha (Plate 2). Yang (1998) [17]. reported post-harvest diseases of gladiolus and Lilium in Taiwan and isolated *Penicillium, Aspergillus, Mucor, Rhizopus, Alternaria, Rhizoctonia solani, Botryosphaeria* and *Fusarium.* He also mentioned their source of infection and how to reduce incidence of post-harvest disease in storage.

Mohammad and Kashi (2005) [12]. isolated *Rhizoctonia* like mycelia from root and stem of gladiolus. Isolated fungi were identified either binucleated or multinucleated *Rhizoctonia* sp. based on hyphal tip characteristic and nuclear number. Binucleate *Rhizoctonia* isolate showed stem and corm rot and mortality symptoms on 35 days old plants, while multinucleate *Rhizoctonia* showed disease during rooting of plant.

Fig 2: Corm rot on Gladiolus Cause by *Rhizoctonia solani*

Leaf spot (*Alternaria alternata*)

Gladiolus found affected by leaf spot disease and the causal fungi was *Alternaria alternata*. The initial symptoms were in the form of small brownish circular spots with yellowish margin on the ventral surface of leaves. Spot at advance stage formed large brownish circular lesion. Spots were coalescing together and covered large area on leaf (Plate 3).

Alternaria alternata is pathogenic on *Glycyrrhiza glabra* it causes leaf spot symptoms reported by Khan and Abdel Karer (1974) [10]. Goyal and Pathak (1982) [8]. observed symptoms of *Alternaria alternata* on *Catharanthus roseus*. Symptoms of disease appeared as small light brown spot involving almost whole of the surface of the leaf.

Pandey and Nigam (1985) [14]. observed symptoms of *Alternaria alternata* on ashwagandha plant with the appearance of small light brown spot on leaves and flowers. Spot coalesces resulted in blight symptoms. In case of dieback disease necrosis of tender twinges from the tip backwards was observed.

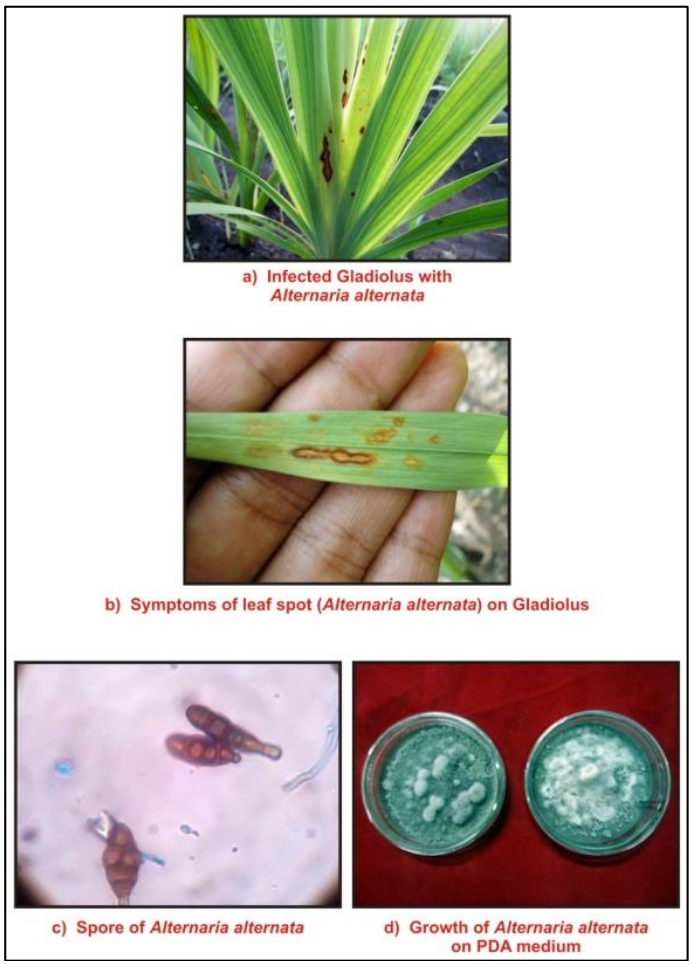

Fig 3: Leaf spot cause by *Alternaria alternata*

Conclusion

Intensive survey was conducted during *Kharif* 2012 to know the incidence of fungal diseases. Gladiolus crop was found infected with fungal diseases i.e wilt (*Fusarium oxysporum* f. sp. *gladioli*), corm rot (*Rhizoctonia solani*) and leaf spot (*Alternaria alternata*).

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