FUNGICIDAL ACTIVITY OF AROMATIC MEDICINAL PLANTS AGAINST *MYROTHECIUMRORIDUM*TODE ASSOCIATED WITH MYROTHECIUM LEAF SPOT DISEASE OF *MOMORDICACHARANTIA* L. (BITTER GOURD)

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ABSTRACT: *MyrotheciumroridumTode emerges as a potential threat for the bitter gourd crop in Pakistan since 2007. Aromatic medicinal plants were applied for its management under greenhouse and field conditions. A total of seven aromatic medicinal plants [garlic (Allium sativum), onion (Allium cepa), ginger (Zingiber officinale), green chilli (Capsicum frutescens), capsicum (Capsicum annuum), turmeric (Curcuma longa) and arvi (Colocasia esculenta)] were intercropped with the bitter gourd for the In vivo evaluation of fungicidal activity. Production technology prescribed by the Punjab Agriculture Department was followed. Ten days old Myrothecium roridum cultures, maintained on potato bitter gourd dextrose agar medium were used as spray (@2x10^{3}spores/ml) for the artificial inoculation on four weeks old seedlings. Among the test plants, garlic, onion and green chilli exhibit a remarkable antifungal potential. Garlic lowers the disease incidence up to 63% under greenhouse and 58% in field experiments followed by the chilies (52% under greenhouseand 49% in field) and onion (41% under greenhouse and 39% in field). Ginger (27% under greenhouseand 22% in field) and capsicum (24% under greenhouseand 17% in field) shows a moderate antifungal potential while turmeric (11% under greenhouseand 5 % in field) and arvi (9% in pots and 8% in field) were least effective. Intercropping of garlic, green chilli and onion can significantly reduce the Myrothecium leaf spot disease incidence in farmer fields. Further studies to evaluate the active chemical constituents responsible for their antifungal potential may help in the development of effective fungicides.*

Keywords: *Myrotheciumroridum*Tode, aromatic medicinal plants, fungicidal activity, *in vivo*.

INTRODUCTION

Vegetable plays an important role in meeting with nutrition needs and fighting against ailments. Among vegetables, bitter gourd (*Momordica charantia* Linn) has a unique medicinal and nutritional value and belongs to family Cucurbitaceae. It is among the popular vegetables in Asia and other part of the world. It is normally grown as an annual crop in Pakistan with the total area under bitter gourd cultivation during 2009-2010 was 6565 hectares and total production of 56994 tones [6]. Bitter gourd fruit is medicinal and nutritious vegetable. The high nutritive value ranks it first among the cucurbits in iron and vitamin C contents. It is an excellent source of phenolic compounds, antioxidants, and antimutagen [5]**.** The fruit has considerable amount of potassium, calcium, magnesium, protein, and dietary fiber as compared with other commercial vegetables [10].

Myrothecium. sp. is soil borne as well as seed borne pathogen and attacks on a wide range of plant species. On cucurbits it causes round dark-brown leaf spot which on later stage coalesces to form blighted areas on the leaves [1]. It requires a prolonged wet period for perpetuation and epidemics. Though *Myrothecium roridum* is frequently observed on above ground parts especially the leaves but it is primarily seed and soil borne in nature. [7] Reported that fungus is associated with rotted and un-germinated seeds. Yield and quality loss of bitter gourd crop is usual phenomenon in Punjab, Pakistan. Periodic occurrence of myrothecium leaf spot disease on the bitter gourd and several other crops belonging to different families and even isolations from gymnosperms needs the detailed study of the pathogen biology, physiology and management.

Application of fungicides is conventional tool for disease management and highly practiced due to effective disease management. Due to health hazards and other economic concerns attention has been diverted for evaluation of alternative strategies for incorporation in Integrated Disease Management (IDM). Study of allelopathic potential of plants against fungal and bacterial pathogens is getting acceptance among the production and consumption chain stakeholders because its cost effectiveness and user safety. Intercropping of different non-host plants with known antimicrobial activity may help in reducing the pathogen build up by providing either physical barriers or releasing volatile chemical constituents that retard the fungal growth. [3] studied the marigold and pigweed allelopathy by intercropping for the management of tomato early blight disease. [8] reported reduced Septoria leaf spot disease by *Septoria lycopersici* in tomato-maize intercropping. Chickpea blight caused by Ascochyta sp was significantly lowered by intercropping chickpea with wheat and barley [2,4].

Little work has been reported in Pakistan on diseases of bitter gourd. The present studies are, therefore, aimed for the management of *Myrothecium* leaf spot disease by intercropping medicinal aromatic plants with bitter gourd crop which will enable us to protect bitter gourd crop from Myrothecium leaf spot disease.

METHODOLOGY

The investigations protocol comprises on greenhouse and field trails. Greenhouse trials were performed at experimental station of institute of agricultural sciences, university of the Punjab (IAGS, PU) Lahore, Pakistan. Field trials were conducted at research forms of plant pathology section, Ayub Agriculture Research Institute (AARI) Faisalabad, Pakistan. Seeds of bitter gourd, chili, capsicum, onion bulbs and rhizomes of turmeric, ginger were procured from vegetable section AARI Faisalabad, Pakistan (Table 1). Single spore culture of *Myrothecium roridum* was isolated from the field grown bitter gourd and maintained on potato dextrose agar medium.

For green house experiments, 18x24 cm earthen pots were used. The pots were filled ¾ with the sandy loam soil while for field experiments; 75-90 cm ridges with 60cm distance between the rows was prepared. Plant to plant distance was maintained at 45cm. Crop Production technology prescribed by the Punjab agriculture department for farmers were followed. The four week old seedling growth was evaluated after the germination of 75% germplasm of all the tested plants. Ten days old *Myrothecium roridum* cultures were sprayed (@2x10^{3}spores/ml) with hand atomizer for artificial inoculation of four weeks old seedlings. The spray was repeated at 30 minutes interval. All the treatments were replicated thrice and each replication contains ten plants. Data regarding disease incidence and disease severity was taken after two days of spray and then at weekly intervals up to 7 weeks (bitter gourd plant maturity) by using the disease rating scale.

Data recording for infection development in pots was initiated 48 hours after inoculation and for field trails inoculation was made at germination, vegetative growth, maturity, harvest and for some selective plants at seed development stage of the plant and data recording was initiated at germination stage. Data recorded was subjected to analysis of variance (ANOVA) followed by students T test using Microsoft excel 2010.

Table 1: Inventory of plants used in intercropping against *Myrotheciumroridum*Tode under greenhouse and field conditions

Plant intercropped	Botanical name	Sowing material
Garlic	*Allium sativum*	Cloves
Onion	*Allium cepa*	Bulb
Ginger	*Zingiber officinale*	Rhizome
Green chili	*Capsicum frutescens*	Seeds
Capsicum	*Capsicum annuum*	Seeds
Turmeric	*Curcuma longa*	Rhizome
Arvi	*Colocasia esculenta*	Cornels

RESULTS

First reading of disease incidence of myrothecium leaf spot under greenhouse was recorded after 48 hours of artificial inoculation as pinhead sized infections and then at weekly intervals up to 7 weeks (Fig.1). The initial disease incidence readings (ranged 17.33-26.07) did not significantly differ among the intercropped treatments compared to the bitter gourd monoculture (control). The final reading taken at week 7 shows a remarkable reduction in disease incidence against control (79.18). Disease incidence in B-Gar treatment was 29.2% followed by B-Chi (38.20%) and B-Oni (46.66%) treatments. B-Gin treatment exhibited 57.62%, B-Cap 60%, B-Cur 70.03% and B-Col treatment disease incidence was 71.84%. under greenhouse conditions during 2013. All the readings are mean values of three replicates and each replication contains ten plants. The readings are subjected to ±5% SE.

Under field conditions, first reading of disease incidence of myrothecium leaf spot was recorded after 48 hours of artificial inoculation as pinhead sized infections (Fig. 2). The initial disease incidence readings (ranged 18.63-36.08) less significantly differ within the intercropped treatments compared to the bitter gourd monoculture (control). Further readings were taken at weekly intervals. The final reading taken at week 7 shows a remarkable reduction in disease incidence against control (78.96). Disease incidence in B-Gar treatment was 33.1% followed by B-Chi (40.29%) and B-Oni (48.19%) treatments. B-Gin treatment exhibited 61.62%, B-Cap 65.75%, B-Cur 75.08% and B-Col treatment disease incidence was 72.43%.

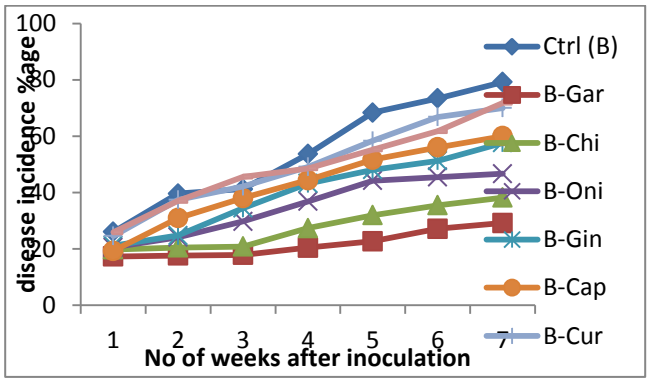

Fig. 1: Disease incidence percentage of Myrothecium leaf spot disease in intercropping treatments

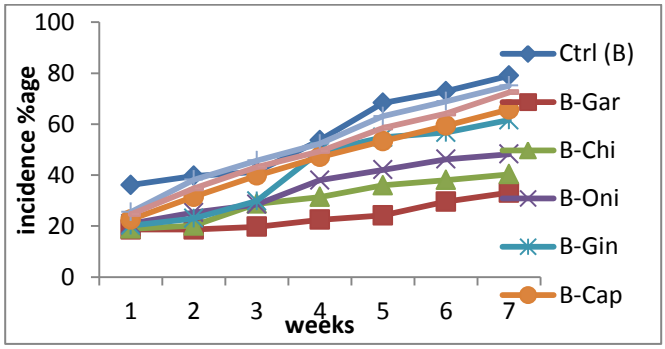

Fig. 2: Disease incidence percentage of Myrothecium leaf spot disease in intercropping treatments under field conditions during 2013. All the readings are mean values of three replicates and each replication contains ten plants. The readings are subjected to ±5% SE.

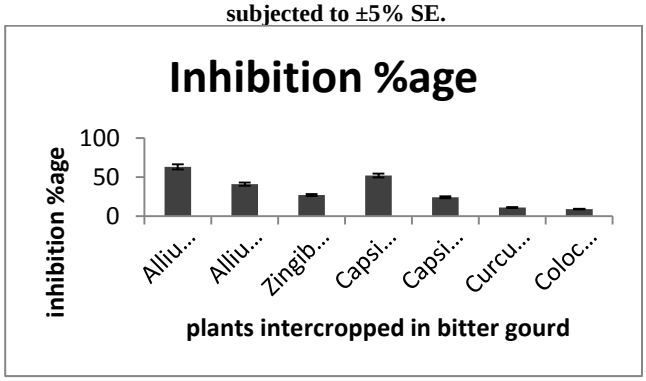

Fig. 3: Disease inhibition percentage of Myrothecium leaf spot disease in intercropping treatments under greenhouse conditions during 2013. All the readings are mean values of three replicates and each replication contains ten plants. The readings are subjected to ±5% SE.

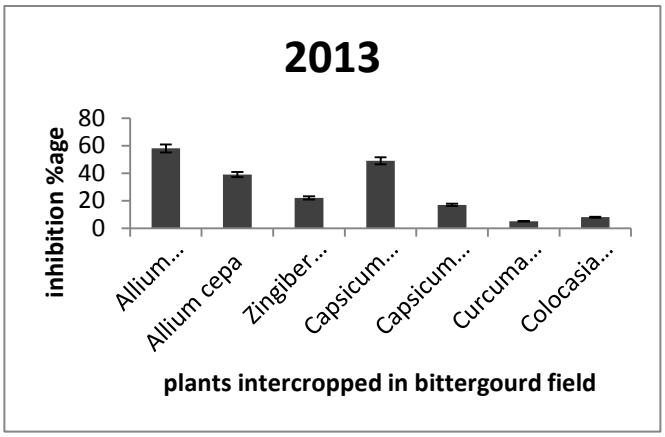

Fig. 4: Disease inhibition percentage of Myrothecium leaf spot disease in intercropping treatments under greenhouse conditions during 2013. All the readings are mean values of three replicates and each replication contains ten plants. The readings are subjected to ±5% SE.

Under greenhouse conditions @($P < 0.05$), bitter gourd-garlic intercropping treatment significantly lowers the incidence of myrothecium leaf spot disease by 63% over control (Fig. 3). Chilies-bitter gourd intercropping also reduce the myrothecium leaf spot disease incidence, 52%, significantly followed by onion-bitter gourd intercropping that shows an inhibition percentage of 41 than the control treatment. Bitter gourd-ginger and bitter gourd-capsicum intercropping treatments were less significantly inhibit the myrothecium leaf spot disease incidence i.e., 27% and 24% respectively. Bitter gourd-turmeric and bitter gourd-arvi intercropping treatments reduce the disease incidence non-significantly by 11% and 9% respectively than the bitter gourd alone.

Under field conditions, the results were much similar to those with greenhouse conditions except for their degree of inhibition slightly decrease. Bitter gourd-garlic intercropping treatment significantly ($P < 0.05$) lowers the incidence of myrothecium leaf spot disease, by 58% than in bitter gourd solo cultivation (Fig. 4). Chilies-bitter gourd intercropping also reduce the myrothecium leaf spot disease incidence, 49%, significantly followed by onion-bitter gourd intercropping that shows an inhibition percentage of 39 than the control treatment. Bitter gourd-ginger and bitter gourd-capsicum intercropping treatments were less significantly inhibit the myrothecium leaf spot disease incidence i.e., 22% and 17% respectively. Bitter gourd-turmeric and bitter gourd-arvi intercropping treatments reduce the disease incidence non-significantly by 5% and 8% respectively than the bitter gourd alone.

DISCUSSION

Intercropping with different aromatic and medicinal plants can lead to mutual economically cost effective as well as environment friendly approach in adopting the production technology of the crop of interest [3]. These may involve the nutrient uptake processes, creating microclimate or escaping the disease either as non-host crop or liberating chemical constituents to fight with the pests [2]. Aromatic plants are well known for their characteristics release of several volatile compounds in their surroundings. Most of these compounds have been reported activity as antimicrobials. They may be used for the management of several insects, bacteria, fungi and nematodes. Garlic and onion are well-known to release allicin compound belonging to solfoxide class that is active against fungi and bacteria [4]. Curcumin, a terpenoid; is reported in turmeric and is biologically active against fungi, bacteria and protozoa. Present studies show that garlic and onion intercropping significantly lowers the disease incidence of myrothecium leaf spot disease of bitter gourd as compared to the monoculture bitter gourd. This reduction might be contributed towards the accumulation of volatile constituents on the plant surface that retard the further infections, sporulation and development of fungus [9]. The results suggest that intercropping of garlic, green chili and onion can significantly reduce the Myrothecium leaf spot disease incidence in farmer fields without increasing their input cost in the form of fungicides. Further studies for evaluation and extraction of the active chemical constituents responsible for their antifungal potential may help in the development of effective fungicides.

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