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Research Article

MYCOFLORA OF *TRIANTHEMA PORTULACASTRUM L.*, AN INVASIVE WEED OF ANDHRA PRADESH

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A B S T R A C T

Trianthema portulacastrum L., a member of aizoaceae is indigenous to South Africa but is widely distributed in tropical and subtropical areas as a noxious weed. The mycoflora namely *Alternaria alternata* (Fr.) Keissler., *Colletotrichum capsici* (Syd.) E.J. Butler & Bisby., *Bipolaris maydis* (Y.Nisik. & C.Miyake) Shoemaker., *Curvularia lunata* (Wakker) Boedijin., *Curvularia tuberculata* Sivan. and *Gibbago trianthemae* E.G. Simmons was isolated from highly infected portions of the weed. The pathogenesis of fungal isolates was confirmed by Koch's postulates primarily and the host specificity of the isolates was evaluated on green house plants by spore treatment. Among the isolates, *Gibbago trianthemae* was highly aggressive to weed and it was considered as potential biocontrol agent (Mycoherbicidal agent).

INTRODUCTION

Trianthema portulacastrum L. (horse purslane - Aizoaceae) is a branched, prostrate, succulent, annual herb indigenous to South Africa (Adamson, 1962; Jeffrey, 1960, 1961) but is widely distributed in Northern India and several other tropical and subtropical areas including West Asia, Africa and Tropical America as an invasive weed of cultivated fields and wastelands (Duthie, 1960; Holm *et al.,* 1997). It is considered as a major weed in various food and vegetable crops such as *Brassica* sp. (Mustard), *Zea mays* L. (Corn), *Cajanus cajan* (L.) Millsp. (Pigeonpea), *Glycine max* (L.) Merr. (Soybean), Solanum *lycopersicon* L. (Tomato), *Solanum tuberosum* L. (Potato), *Allium cepa* L. (Onion) and *Gossypium hirsutum* L. (Cotton). It has become a noxious weed due to competition for yields in many crops like *Pennisetum glaucum* L. (Millet), *Sorghum bicolor* L. (Sorghum), *Zea mays* L. (Maize), *Triticum aestivum* L. (Wheat), *Vigna mungo* L. (Mash), *Vigna radiata* L. (Mungbean), *Cyamopsis tetragonoloba* L. and *Helianthus annuus* L. (Sunflower) and causing significant reduction in the yield (Nayyar *et al.,* 2001). Up to 60 - 70% infestation of this weed has been reported in pigeon pea and soybean fields and 80-90% in *Maize* and *Brassica* fields (Aneja *et al*., 2000). Horse purslane is a harmful weed infested in many vegetable crops like brinjal, okra and other vegetables. The control of horse purslane in field crops is very essential due to the increase of loss in yield of many crops in every year and also many farmers depended on these food and vegetable crops for their economy. The weed, horse purslane is currently controlled by mechanical methods and also the application of pre - and post - emergence herbicides such as acifluorfen, alachloral, atrazine, bentazon, fluchloralin, fomesafen, paraquat and pyrivate. But in view of pesticide residues and environmental pollution, the exploitation of microorganisms especially plant pathogenic fungi is now emerging as an effective and eco-friendly alternative to conventional methods of weed control (Charudattan, 1991). Mycoherbicides are attractive agents in weed management because of their specificity, low environmental impact and cost effective (Bohra *et al.,* 2005; Boyette *et al.,* 2007). Many kinds of pathological symptoms on *Trianthema portulacastrum* at field conditions reported by a systematic epidemic study conducted at agricultural fields of Visakhapatnam District, Andhra Pradesh during 2012-2013. The weed population in natural conditions was extensively suppressed by natural enemies such as fungal pathogens which cause foliar symptoms like leaf spots, leaf blights, necrosis and defoliation. Moreover, the wilting of stems was observed at mature stage of various symptoms. In view of the above the research was aimed to screening of mycoflora and their pathogenesis against horse purslane weed to develop mycoherbicide agents.

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MATERIALS AND METHODS

Field study and sample collection

The field observations on infestation of horse purslane were conducted in different agricultural crops classifieds as food

crops, pulses, vegetable crops, oil crops and commercial crops at Vishakhapatnam District (Fig 1). The weed infestation was studied using random sampling method in all agricultural fields and some valuable information about the weed infestation was gathered from local formers. The parasitized leaves and various symptoms on horse purslane were critically studied during 2012-2013 at field sites and photographs of diseased leaves and whole plants were taken using digital camera.The diseased plants and propagules were collected randomly into sterilized polythene bags and brought to the laboratory for the extensive study on symptoms, isolation and virulence of the organism (s) involved in pathogenesis. The disease symptoms on leaves and stems and other plant parts were critically examined in plant pathology laboratory, Department of Botany, Andhra University, Visakhapatnam.

Screening of mycoflora

Culture preparation

The diseased leaves were washed thoroughly in running tap water to remove soil particles and the infected portions of the leaves were cut into 1.0 1.5 cm fragments. The pieces were surface sterilised by 70% ethyl alcohol for 1-2 minutes and then rinsed in sterile distilled water for three to four times. Finally the leaf bits were rinsed in 0.01% mercuric chloride for 1 or 2 minutes fallowed by washing with sterile autoclaved double distilled water for 2 or 3 times. These fragments were transferred on to Czapek's Dox Agar (CDA) and Potato dextrose agar (PDA) plates supplemented with 1% streptomycin sulphate (antibiotic) under sterile conditions in an inoculation chamber (Fig 2). After inoculation plates were incubated at 25 ±2°C for 21 days under a 12 h light/dark photoperiod.

Isolation and identification of mycoflora

The isolates were purified during the initial growth of fungal colonies on inoculated leaf lesions on the surfaces of agar media. The stock cultures of the isolates were prepared using mono culture (single conidial culture) and stored at room temperature as slant cultures on PDA media. The isolates were examined by the staining techniques and diagnostic characteristics of the isolates were examined under light microscope. The identification features of each isolates such as colony diameter, colour, texture, sporulation, secondary metabolites, the shape and sizes of conidiophores and conidia were carefully studied. Identification of the fungal isolates was made with help of the relevant literature (Barnett, 1960; Barron, 1968; Domsch *et al.,* 2007; Ellis, 1971, 1976; Gilman, 1957; Holliday, 1993; Nagamani *et al.,* 2006; Sivanesan, 1987; Sutton, 1980; Simmons, 1986; Mitchell, 1988; Aneja and Kaushal, 1998; Aneja *et al.,* 2000).

Spore treatment experiments

Spore inocula of isolates were harvested from 14 d old cultures incubated at 25 ± 2° C with a 12 h light/dark photoperiod. Conidia and mycelium production were carried out on young sporulated cultures of the isolates in aseptic conditions. The finest spore inocula (10^4-10^6 spores/ml) were made using sterilized spatula by flooding the plates with sterile 20 ml distilled water and then scraping the mycelial mass slowly for conidial suspension. Afterthat, the suspension was filtered through sterile, muslin cloth folded in four layers and the final inoculum was taken into 100 ml conical flasks containing sterile distilled water mixed with 0.02% (v/v) Tween 20 (Merck), the wetting agent. The Inoculum concentration was adjusted to $1x10^4$ to $5x10^4$ spores/ml using Improved Neubauer haemocytometer (Depth = 0.1mm).

Green hose conditions and Experiments

Seeds and seedlings of horse purslane (*Trianthema portulacastrum* L.) were collected from agricultural fields during the field study. The collected seeds were dried and maintained in healthy condition without any contamination. The plants for further studies were grown by sowing the seeds in 25x15 cm diameter plastic pots containing sterilized black soil. The pots containing seedlings of weed plants were maintained at 25-30^{O} C on wood stand in a green house with a 12 h light/dark photoperiod. For host range studies test plants were maintained in five replicates (10 plants for each replicate) along with control plants. The test plants growing in aseptic greenhouse conditions were watered at healthy conditions. Infected plants observed at pre-inoculation stage were avoided from pathogenicity test. The test plants with healthy, young and greenish leaves were used for the spore inoculation of the fungal isolates.

Disease intensity (DI)

The intensity of infection was determined visually based on the initiation of disease and increase in disease area on the leaves and stems of the test plants every day. Spore inoculum was applied onto the test plants of *Trianthema portulacastrum* within 2 hours of sunset to avoid drying and to allow for a natural dew period shortly afterwards. Plants were observed three days after treatment (DAT) for disease symptoms. The intensity of infection was determined visually based on the number of infected leaves or area of infected parts or whole plants. The disease intensity on leaf surfaces and the development of symptoms were observed daily. The disease intensity of pathogens on test plants was determined using a score chart (-, no symptoms, a healthy plant; +, mild symptoms, a plant showing slight symptoms on 15% of the leaf area; ++, moderate symptoms, a plant showing definitely bigger patches of diseased areas on 16 to 59% of the leaf area; and +++, severe symptoms, enlarged lesions covering 60 to 80% of the leaf area) (Ray and Hill, 2012).

RESULTS AND DISCUSSION

In field study the natural infection on horse purslane leaves and stems were observed in various locations of field area. The typical fungal symptoms on parasitized parts of the horse purslane were noticed. The symptoms on leaf surfaces were examined as leaf spots, leaf blights, necrosis and defoliation. Moreover, the wilting of stems was observed at mature stage of various symptoms caused by fungal pathogens. Foremost, in laboratory conditions the pathogens were isolated from leaf lesions of naturally infected horse purslane plants. A total of six isolates namely *Alternaria alternata* (Fr.) Keissler., *Colletotrichum capsici* (Syd.) E.J. Butler & Bisby., *Bipolaris maydis* (Y.Nisik. & C.Miyake) Shoemaker., *Curvularia lunata* (Wakker) Boedijin., *Curvularia tuberculata* Sivan. and *Gibbago trianthemae* E.G. Simmons (1986) were identified in cultures of horse purslane parasitized leaf bits (Table 1). The macroscopic characteristics such as colony diameter, colour, texture, and sporulation observed by culture plate technique while the microscopic characteristic of each isolate was studied using different features of conidiophores,

conidia and fruit bodies, and spore germination under light microscopy (Fig 3).

Table 1 Fungi isolated from infected parts of *Trianthema portulacastrum* L.

Fungus Name	Isolated part
Alternaria alternata (Fr.) Keissler.	Leaf
Bipolaris maydis (Y.Nisik. & C.Miyake) Shoemaker.	Leaf
Curvularia lunata (Wakker)Boedijin.	Leaf
Curvularia tuberculata Sivan.	Leaf
Colletotrichum capsici (Syd.)E.J.Butler & Bisby	Leaf
Gibbago trianthemae E.G. Simmons (1986)	Leaf, stem & petiole

Alternaria alternata (Fr.) Keissler

The fungus produced profuse mycelial growth on PDA. Initially the mycelium was hyaline that turned to grey-brownish, multicelled, septate and irregularly branched. In early growing stage hyphae were thin, narrow, and hyaline but became slightly thick as they grew old. Conidiophores arised singly or in clusters, usually 2-6 and were long or short. They were pale olivaceous to olivaceous - brown, straight or curved, geniculate, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. Conidia were in chains, light olivaceous to dark brown, septate and muriform.

Colletotrichum capsici (Syd.) E.J.Butler & Bisby

The isolate was identified based on size and shape of conidia. Isolate produced cottony colonies on PDA with a colour of greyish - to dark grey on the ventral surface whereas the reverse of the colonies was mainly black. The colony diameter ranged from 65 to 70 mm after 10 days incubation. Conidiophores unicellular, hyaline, cylindrical, phialidic, septate, sometimes branched, tapered towards the apex, 20 µm long and 3 µm wide. Conidia formed in white masses, one-celled, smooth walled, hyaline, falcate, tapering towards each end with acute apex and truncate base.

Bipolaris maydis (Y.Nisik. & C.Miyake) Shoemaker

Colonies appeared black to greyish black in PDA; conidia relatively long and broad with dark brown colour, slender and slightly curved; Conidiophores brown, producing conidia through an apical pore and forming a new apex by growth of the subterminal region; conidia fusoid, straight or curved, germinating by one germ tube from each end. The identification features of the isolates include the shape and colour of conidiophores and conidia. Conidiophores mid - to dark brown in colour, medium to long, commonly long, slender, straight or curved, single or in groups of 2 or 3, pale near the apex, smooth, up to 700 µm long, and 5-10 µm thick, and bear conidia at wide intervals. Conidia are distinctly curved, broad in the middle, sharply tapering towards rounded ends, pale to mid-dark golden brown, smooth, 5-11 septate, mostly 70-160 µm long, 15-20 µm thick in the broadest part.

Curvularia lunata (Wakker) Boedijin

Colonies blakish brown; Conidiophores maco or mononematous, unbranched, terminal, often geniculate above, sympodial, cylindrical; conidia acropleurogenous, straight, ovoid, obclavate or ellipsoidal, unequal sides or rarely with slight curvature, 3-5 mostly 3 - septate, middle cells darker, end cells subhyaline to pale or dark brown, mature conidia tuberculate, 23x-52x13-20 µm, young conidia subhyaline and smooth walled.

Curvularia tuberculata Sivan

Colonies on PDA dark gray, usually zonate; Colonies on natural substrat effused, brown to black, hairy; mycelium on natural substrate usually immersed; hyphae branched, septate, colorless or brown, smooth or verrucose. Conidia acropleurogenous, sometimes in whorls, arise through pores in the conidiophore wall, straight or curved, usually broadly fusiform, ellipsoidal, obovoid, clavate or pyriform, sometimes rounded at the base, sometimes with a distinctly protuberant hilum, septate, often with one or more cells larger and darker than the others, smooth or verrucose.

Gibbago trianthemae E.G. Simmons

Subsurface mycelia growth was dense and dark on PDA, and inconspicuous on TWA. Sporulation was excellent at agar surfaces of ***Czapek*** Dox Agar and the moderate amounts of sporulation appeared on PDA with woolly aerial mycelium. Conidia produced in culture were characterized by means of secondary conidiophores. Conidiophores simple or 1-2 proliferated. 1-4 transeptate, pale straw- colored, up to 60-80 x 5-6 µm, very slightly swollen at apex, producing a solitary conidium at the apex of each proliferation, retaining a distinct umbilicate or crateriform depression at the conidiogenous locus after secession of conidium. Conidia initially solitary, almost perfectly ellipsoid; becoming broadly ellipsoid to broadly sub ovate -ellipsoid, with 1-4 complete or partial transverse septa.

Pathogenicity of the isolates

An *in vitro* test was carried out to confirm the pathogenicity of isolates on horse purslane plants growing in green house conditions (Table 2).

Table 2- Pathogenicity of isolates on horse purslane weed after $5x10^4$ spores/ml inoculum concentrations

Isolate	Disease intensity	Symptoms
Alternaria alternata (Fr.) Keissler.	++	Minute leaf spots & necrosis
Bipolaris maydis (Y.Nisik. & C.Miyake) Shoemaker.	+	Minute leaf spots
Curvularia lunata (Wakker)Boedijin.	–	No symptoms
Curvularia tuberculata Sivan.	-	No symptoms
Colletotrichum capsici (Syd.)E.J.Butler & Bisby	-	No symptoms
Gibbago trianthemae E.G. Simmons (1986)	+++	Leaf spot, leaf blight & stem wilt

Disease intensity (score chart):

- \- = no symptoms, a healthy plant;
- \+ = mild symptoms, a plant showing slight symptoms on 15% of the leaf area;
- ++ = moderate symptoms, a plant showing bigger patches on 16- 59% of leaf area;
- +++ = severe symptoms, a plant showing enlarged lesions covering 60 to 80% of the leaf area

The test plants inoculated with spore concentrations ($5x10^4$ spore/ml) of each isolate were examined at 24 h after the treatment. The disease intensity was measured in terms of disease intensity and the results were analyzed. The isolates namely *Alternaria alternata* (Fr.) Keissler., *Bipolaris maydis* (Y.Nisik. & C.Miyake) Shoemaker. Produced moderate symptoms on leaves of horse purslane at 5 d after inoculation. The phaeodictyoconidial fungus *Gibbago trianthemae*

Simmons infected extremely on leaves and stems of the test plants and produced typical symptoms which were similar to field observations. The fungal pathogen *Gibbago trianthemae* was reisolated from the infection areas of the inoculated plants and the pathogenicity of the isolate was confirmed on host plant.

CONCLUSION

The infestation of *Trianthemae portulacastrum* L. was studied in the field crops of Visakhapatnam District of Andhra

Figure 1 Collection of weed samples at Chodavaram Mandal of Visakhapatnam, Andhra Pradesh

The remaining isolates namely *Colletotrichum capsici* (Syd.) E.J.Butler & Bisby., *Curvularia lunata* (Wakker) Boedijin. and *Curvularia tuberculata* Sivan considered as non-pathogenic to horse purslane which were failed to produce disease symptoms and eliminated from epidemic studies. *G. trianthemae*, the foliar fungal pathogen of horse purslane was harvested from maroon coloured lesions inoculated on the surface of the growth media. After green house experiments ($5x10^4$ ml^{-1} spore treatment), the symptoms caused by *G.trianthemae* on inoculated leaves started after 3-4 days of spore treatment. Initially symptoms were pin-point, with maroon margins up to 1 mm in diameter. The lesions became sunken and cause necrosis after 7-9 days of inoculum spraying. The infected leaves became chlorotic followed by defoliation very soon (Fig 2 & 3).

Figure 2 Pure cultures of fungal species on PDA media

Under severe attack quite similar lesions were also examined around the stems causing withering. In earlier, the pathogenesis of *G. trianthemae* on horse purslane was reported by various workers. However, the evolution of mycoherbicide properties of the isolate *G.trianthemae* not reported widely excluding in USA and India. (Simmons, 1986; Mitchell, 1988; Aneja and Kaushal, 1998; Aneja *et al.,* 2000).

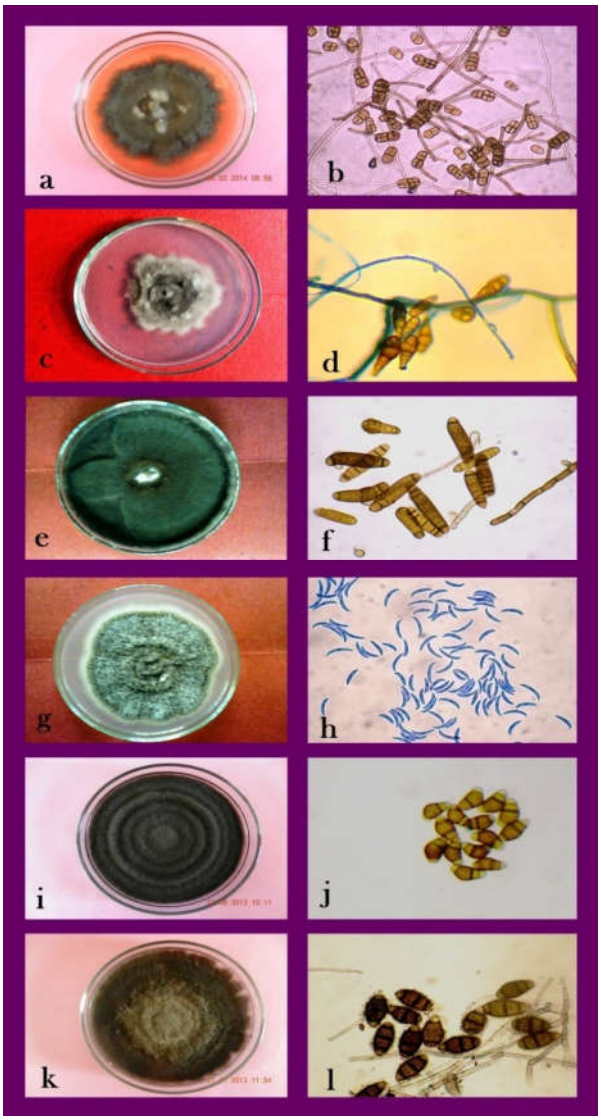

Figure 3 Macroscopic (colony diameter) and Microscopic (spores) features of fungi isolated from diseased leaves of horse purslane (a&b) *Gibbago trianthemae* (c&d)*Alternaria alternata* (e&f) *Bipolaris maydis* (g&h)*Colletotrichum capsici* (i&j) *Curvularia lunata* (k&l)*Curvularia tuberculata*

Pradesh. During the field study horse purslane was heavily competing with agricultural crops such as paddy, jowar, maize, sugarcane, groundnut, brinjal, tomato and okra etc. The maximum weed infestation was recognised in the vegetable crops such as okra, tomato, capsicum and ridge gourd which resulted the heavy loss of crop production. Considering the invasion of horse purslane the effective control methods are very indispensable. In spite of the use of chemical herbicides to control this weed, many adverse effects due to this weed have been reported in various agricultural zones in India. Moreover, the use of herbicides leads to the resistance of wee and environmental pollution by means of pesticides residues in soil, water, and other sources. Therefore, keeping this in mind the eco-friendly weed control method (Biological control), as alternate to chemical herbicides, the investigation was attempted to screening of fungal pathogens as the effective mycoherbicide agents. A total of six fungal species viz., *Alternaria alternata* (Fr.) Keissler., *Colletotrichum capsici* (Syd.) E.J.Butler & Bisby., *Bipolaris maydis* (Y.Nisik. & C.Miyake) Shoemaker., *Curvularia lunata* (Wakker)Boedijin., *Curvularia tuberculata* Sivan. and *Gibbago trianthemae* E.G. Simmons (1986) were isolated and identified on infected plants of *Trianthema portulacastrum* L.(horse purslane), the dominant weed in many agricultural crops in study area. The isolates namely *Alternaria alternata* and *Bipolaris maydis* were shown moderate symptoms on host plant while the pathogenicity of other species such as *Colletotrichum capsici, Curvularia lunata* and *Curvularia tuberculata* was not recorded and they were considered as non pathogenic fungi to horse purslane weed. Our findings revealed that *Gibbago trianthemae* caused severe infection on host weed within short span of time. Our study suggested that *G. trianthemae* to be a potential agent to horse purslane and the extensive work in field conditions was needed to justify the virulence of the pathogen on host weed. We reported the primary information on pathogenicity of *G.trianthemae* against *T. portulacastrum* at crop fields of Visakhapatnam District and the extensive study on host range, the host-pathogen interaction, infection process, growth and sporulation, mass culture and compatibility with various pesticides is indispensable for the development *Gibbago trianthemae* as an effective mycoherbicide.

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