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Short communication

Isolation, host specificity and biocontrol potential of *Gibbago trianthemae* against horse purslane weed

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Trianthema portulacastrum L. (Aizoaceae) has been considered as one of the invasive weeds in Visakhapatnam District and has become an important weed due to its significant interference with many agricultural crops like brinjal, okra and other vegetable crops. The weed is currently controlled by mechanical methods and also the application of pre- and post-emergence herbicides. 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 effectiveness (Bohra *et al.* 2005, Boyette *et al.* 2007). The fungal species *G. trianthema*e on *Trianthema* weed was first recorded from India by Aneja and Kaushal (1998). Earlier it was described from the USA, Cuba and Venezuela as a new phaeodictyoconidial genus (Simmons 1986). Several workers reported that *G. trianthemae* is pathogenic to *T. portulacastrum* (horse purslane) and it may be a possible candidate for inundative mycoherbicide to control horse purslane. (Aneja *et al.* 2000, Akhtar *et al.* 2013).

Weed infestation was studied using a random sampling method from different agricultural fields at Vishakhapatnam District. The diseased plants and propagules were collected randomly into sterilized polythene bags and brought to the laboratory for further study. 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 followed by washing with sterile autoclaved double distilled water for 2 or 3 times. After surface sterilization, the leaf bites were transferred on to Czapek's Dox Agar (CZA) and potato dextrose agar (PDA) plates supplemented with 1% streptomycin sulphate (antibiotic) under sterile conditions in an inoculation chamber. After inoculation, plates were incubated at 26 ±2°C for 21 days under a 12 h light/dark photoperiod in an incubation chamber. The isolates were purified from the fungal colonies sprouting on inoculated leaf lesions. The stock cultures of the isolates were prepared by monoculture (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.

Seeds of *T. portulacastrum* were collected from the plants. The collected seeds were dried and maintained in healthy conditions. The plants for further studies were grown by sowing the seeds of horse purslande in 25 x 15 cm diameter plastic pots containing sterilized black soil. The pots containing seedlings of weed plants were maintained at 25-30^{0}C on wood stand in a green house with a 12 h light/dark photoperiod. For host range studies, test plants were maintained in replicates along with control plants. The test plants with healthy, young and greenish leaves were used for the spore inoculation of the fungal isolates. Spore inocula harvested from young colonies of the isolates was adjusted as précised concentrations (5x10^4 spores/ml) using improved Neubauer haemocytometer (Depth = 0.1 mm) for the spore treatment of test plants and other plants for host range (**Table 1**). The spore application on greenhouse test plants was carried out by using hand spryer and 0.02% of Tween-20 was added to spore suspension as an adjuvant. Spore inoculum was applied onto the test plants of *T. portulacastrum* and host range plants 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.

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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) following methods of Ray and Hill (2012).

Field observations

Horse purslane was found severely competing with agricultural crops such as paddy, sorghum, maize, sugarcane, groundnut, brinjal, tomato, ladies' fingers *etc*. The maximum weed infestation was found in the vegetable crops such as ladies' fingers, tomato, capsicum and ridge gourd. The typical fungal symptoms on parasitized parts of the horse purslane were noticed. Disease symptoms on leaves were found as pinpoint round to oval maroon spots in early stages of infection. The disease symptoms were observed on both adaxial and abaxial leaf surfaces. The diseased spots expanded with the passage of time and became sunken and straw coloured with maroon borders. Later, few spots coalesced and the whole leaves became chlorotic and dried up causing severe defoliation. Under severe attack quite similar lesions were also observed around the stems causing withering. Although all the stages of leaves showed infection, the mature leaves were more heavily affected.

Pathogenicity and host range

A total of five isolates namely *Alternaria alternata* (Fr.) Keissler., *Bipolaris maydis* (Y.Nisik. and C. Miyake) Shoemaker., *Curvularia lunata* (Wakker) Boedijin., *Curvularia tuberculata* Sivan. and *Gibbago trianthemae* Simmons were identified in cultures of horse purslane infected leaf bits but only the isolate *G. trianthemae* was confirmed by pathogenicity tests as the causal agent of leaf spot on host weed.

A total of 50% test plants of horse purslane were infected within 20 days of spore treatment. *G. trianthemae* caused severe wilting and chlorosis of test plants of horse purslane within 30 days of inoculation. Defoliation followed by mortality of horse purslane was observed between 35 to 40 days of post inoculation (**Table 2**). No pathogenic symptoms were visible on crop plants included *Zea mays* L., *Eleusina coracana* Gaertner, *Cajanus cajan* (L.) Millsp., *Vigna mungo* (L.) Hepper, *Solanum melongena* L., *Abelmoschus esculentus* (L.) Moench, *Lycopersicon esculentum* Miller., *Capsicum annuum* L., *Arachis hypogaea* L., *Sesamum indicum* L., *Amaranthus viridis* L., *Brassica oleraceae* L. and *Spinacia oleraceae* L. Test revealed that isolate of *G. trianthemae* caused extensive damage to host plant (horse purslane) only and was not harmful to crop plants (**Table 1 and 2**).

However, some studies have indicated that host-specific biocontrol agents can also exhibit significant damage to non-target effects (Pearson and Callaway 2003). Our study findings suggested rhat pathogen has virulence against its host plant (horse purslane) only and not harmful to other economically important crop plants tested *in vitro*. To date, the pathogen has not been found to cause disease on any other plant species. This paper has reported only primary information on pathogenicity of *G. trianthemae* against *T. portulacastrum* but extensive studies on host-pathogen interaction, infection process, growth and sporulation, mass culture and compatibility with various pesticides are needed for the development of effective mycoherbicide.

Table 1. Host specificity of *G. trianthemae* after spore treatment on test plants

Plants used for spore treatment	Family	DI	Disease reaction
Control plant			
Trianthema portulacastrum L.	Aizoaceae	+++	**S**
Test plants			
Zea mays L.	Poaceae	-	R
Eleusina coracana Gaertner	Poaceae	-	R
Cajanus cajan (L.) Millsp.	Fabaceae	-	R
Vigna mungo (L.) Hepper	Fabaceae	-	R
Solanum melongena L.	Solanaceae	-	R
Abelmoschus esculentus (L.) Moench	Malvaceae	-	R
Lycopersicon esculentum Miller.	Solanaceae	-	R
Capsicum annuum L.	Solanaceae	-	R
Arachis hypogaea L.	Fabaceae	-	R
Sesamum indicum L.	Pedaliaceae	-	R
Amaranthus viridis L.	Amaranthaceae	-	R
Brassica oleraceae L.	Brassicaceae	-	R
Spinacia oleraceae L.	Chenopodiaceae	-	R

DI= Disease intensity; +++ = Severe infection; - =No infection; S= Susceptible; R=Resistant

Table 2. Disease intensity of *Gibbago trianthemae* at different growth stages of horse purslane weed

Concentration of inoculum	Growth stage	PDI
5 x 10^4/ml + 0.02 (Tween-20)	Stage -1 (3-5 foliage)	95.5 ±1.1
	Stage -2 (6-10 foliage)	87.04± 2.0
	Stage -3 (11-14 foliage)	78.9±3.2
	Stage -4 (15-20 foliage)	77.08±2.4

PDI= Percent Disease Index (Mean ± Standard Error)

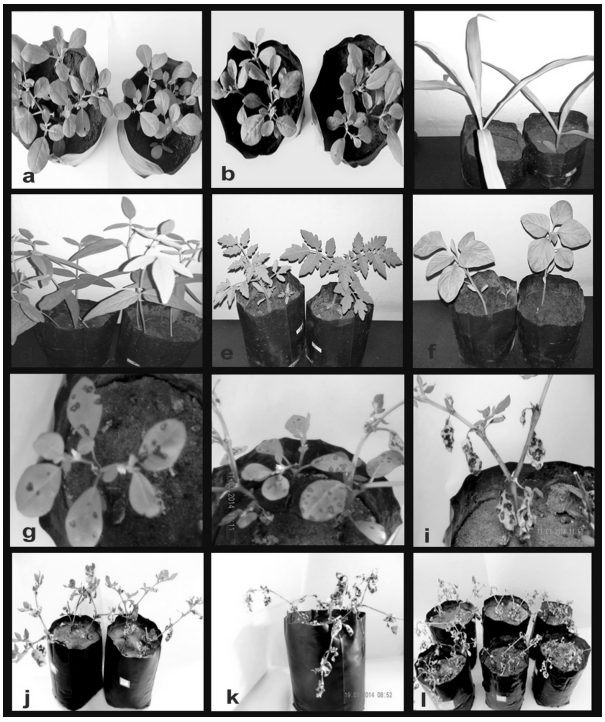

Figure 1. *In vitro* test on host specificity of *G. trianthemae*

A&B-Control plants of horse purslane;C, D, E & F - test plants *Zea mays*, *Cajanus cajan*,*Lycopersicon esculentum* and *Amaranthus viridis* respectively;G, H & I - Development of leaf spot on host weed; J & K- Defoliation of host weed;I - Eradication of horse purslane weed

SUMMARY

In vitro study, pathogenicity test of the isolate, *Gibbago trianthemae* which causes leaf spot and blight disease on horse purslane was confirmed. Study revealed that *G. trianthemae* is highly pathogenic to horse purslane as evidenced by the rapid rate of infection and colonization of the host. The test plants inoculated with 5x10^4 spores/ml concentration showed high susceptibility to *G. trianthemae* incheding of crops only. Foliar pathogen *G. trianthemae* might be useful as a mycoherbicide to control its host weed.

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