
Management of Horse purslane (*Trianthema portulacastrum* L.) with *Gibbago trianthemae* Simmons in India

K. R. ANEJA, S. A. KHAN, and S. KAUSHAL

Department of Microbiology, Kurukshetra University,
Kurukshetra – 136 119 Haryana (India)

Abstract

Horse purslane (*Trianthema portulacastrum* L., Family Aizoaceae), is an introduced terrestrial weed in India. It has become a noxious weed due to competition for yields in various agricultural and vegetables crops such as mustard, maize, pigeon pea, soybean, potato and onion crops in northern India. 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. Between 1989 and 1998 a series of surveys of plant pathogenic fungi associated with naturally infected horse purslane were conducted in the states of Haryana and Punjab. Infected leaves collected from various sites, yielded a species of *Gibbago*, identified as *Gibbago trianthemae* Simmons, a phaeodictyoconidial hyphomycetous fungus reported on horse purslane for the first time in India. In experimental pots, defoliation started after 20 days of inoculum spraying. Percent infection on leaves ranged between 72 and 84 percent, 30 days post inoculation with a conidial suspension at concentration of 2.2×10^5 conidia/ml. Application of inoculum significantly reduced the production of leaves, height and biomass per plant as compared to control. Germination of *Gibbago* conidia took place within 6 to 12 hours. Symptoms on leaves initiated as small pin point lesions, 3-4 days after spraying of inoculum. A significant correlation between the growth and sporulation of the pathogen was reported when tested on 10 different culture media. Best sporulation was found on *Trianthema* extract dextrose agar followed by Potato dextrose agar and Potato dextrose agar+yeast extract ($8.6 \times 10^5 > 8.0 \times 10^5 > 7.37 \times 10^5$ conidia/ml, respectively). Fungus showed growth but failed to sporulate in all the three broths. Best sporulation was recorded at 25°C. Conidia germinated from between 15 and 35°C, the best recorded at 25°C. *G. trianthemae* has most of the criteria desirable for development of a biocontrol agent as a mycoherbicide to control horse purslane; it is easily cultured, sporulates well, infection can take place from conidia and/or mycelial fragments.

Introduction

Horse purslane (*Trianthema portulacastrum* L.) is a much branched, prostrate and annual terrestrial weed of the Family Aizoaceae. An indigenous plant to South Africa has been reported to be widely distributed in India, Srilanka, West Asia, Africa and Tropical America (Duthie, 1960, Balyan and Bhan, 1986). In India, horse purslane has been reported in the states of Uttar Pradesh, Punjab, Haryana, Rajasthan and Delhi and considered as a number one problematic terrestrial weed by virtue of its infestation in various agricultural and vegetable crops such as mustard, maize, pigeon pea, mung bean, potato, onion, cotton, soybean, pearl millet and sugarcane, especially during the rainy seasons (Balyan and Bhan, 1986; Simmons, 1986). Although horse purslane is causing heavy losses in yield of agricultural crops, no systemic survey has been conducted on this weed around

the world.

It is currently controlled mechanically and treatment with pre- and post- emergence herbicides. Hand weeding and hoeing are common practices of controlling this weed in the developing countries of the world; but this method is quite expensive and time consuming thus ineffective as new weed seeds germinate after every hoeing and reinfest the crop, thus using maximum soil nutrients. Moreover, hoeing is not possible during rainy season and labor shortage due to paddy transplanting at that time further accentuates the problem (Brar *et al.*, 1995).

Although chemical herbicides are the most effective immediate solution to most weed problems but increased and indiscriminate use of these resulted in resistant and resurgence in pests. Moreover, persistent residues of DDT and HCH highly poisonous to human beings have been found in vegetables, milk, butter, meat as well as in mother's milk. Several herbicides illustrates biological control of weed with fungal pathogens with potential (Schoreder and Muller-Scharer, 1995; Mortensen, 1998; Aneja, 1999). Excepting work of Mitchell (1988) at the University of Arkansas, USA no other work has been done on controlling this weed by biological means around the world.

The purpose of the present study was to survey for the infestation of various agricultural crops and fungal pathogens causing severe symptoms and ultimately reducing the plant population.

Materials and methods

In the years 1989-1998 surveys for the infestation of various agricultural crops and natural enemies of horse purslane were conducted in northern states of India, the Haryana and Punjab. A population of horse purslane in mustard fields at Kurukshetra near the Sthaneshwar Mahadev Mandir, in February 1990, was found heavily affected by a leaf spot disease. Diseased leaves were collected in sterilized polythene bags and brought to the laboratory for study of symptoms, isolation and pathogenicity test of the organism (s) involved.

Isolation of the pathogen

The diseased leaves were washed thoroughly in running tap water to remove soil particles adherent to the leaves. The infected portions of the leaves were cut into 1.0 – 1.5 cm. fragments, surface disinfected in 70% ethyl alcohol for 1-2 minutes and then rinsed in sterile distilled water six to seven times. These fragments were transferred to Potato dextrose agar (PDA) and Trianthema extract dextrose agar (TeDA) plates supplemented with streptomycin sulphate and were incubated at 25±2°C. TeDA medium (Fresh horse purslane leaves 200.0 g; Dextrose 15.0 g; Agar-agar 20.0 g; and Distilled water 1000.0 ml.) was prepared as follows: Fresh horse purslane leaves (200 g) were washed in running tap water and then in distilled water. These were boiled for 20-25 minutes in 500 ml distilled water and filtered through cheese cloth for the collection of extract. The rest of the procedure was similar to the PDA preparation (Aneja and Singh, 1989).

Pathogenicity test

Pathogenicity of the chosen isolate was determined in *in vitro* and *in vivo*.

in vitro pathogenicity tests – healthy leaves of horse purslane were used for inoculation. They were washed with sterile distilled water and wiped with a cotton swab dipped in 70% alcohol. Some of the leaves before inoculation were injured on adaxial surface by

pricking with a flamed needle. Mycelial discs, taken from 5 days old colony, were placed on injured and uninjured portions and covered with sterile moist cotton. The inoculated leaves were kept in sterilized moist chambers and incubated at $25\pm 2^{\circ}\text{C}$. Regular observations for the appearance of symptoms were made after 3 days of incubation.

in vivo pathogenicity test - seeds of horse purslane were grown in 24 earthen pots with 5 seeds/pot. Two sets of pots (i.e. 12 pots/set) were prepared. One set was kept as control, where no inoculum was added. In the another experimental set, inoculum+surfactant (.02% Tween 80) was sprayed twice onto the leaf surfaces using automizer. First inoculation was made seven days after planting when most plants were at the cotyledon to first leaf stage. Second inoculation was carried out 15 days after the first spray. Of the 12 pots, six pots were covered with polythene bags to maintain relative humidity and six were left uncovered. An equal number of controls were also kept so that plants in both sets received all possible combination of inoculated vs uninoculated, covered vs uncovered. Inoculum was prepared by growing the fungus on TeDA plates and mycelial and conidial mass was harvested by flooding the plates with sterile distilled water and then scraping the mass with a sterilized spatula. Inoculum concentration was adjusted to 2×10^5 spores/ml using hemocytometer. Inoculum was applied to the plants within 2 hrs of sunset to avoid drying and to allow for a natural dew period shortly afterwards.

Plants were observed weekly for the development of disease symptoms. Disease was calculated as percent leaf number infection, 15 days and 30 days post inoculation and t-test was used to indicate the differences between means.

Host range studies

Test plants were selected based on the centrifugal phylogenetic relationship with the target weed (Wapshire, 1974) as well as on the basis of their economic importance. The host species include Pig weed (*Amaranthus viridis*), Bathua (*Chenopodium album*), Wheat (*Triticum aestivum*), Maize (*Zea mays*), Jawar (*Sorghum vulgare*), Rice (*Oryza sativa*), Oat (*Avena sativa*), Barley (*Hordeum vulgare*), Mustard (*Brassica campestris*), Arhar (*Cajanas cajan*) and Soybean (*Glycine max*).

Plants were grown in plastic pots (5 seeds/pot) and were inoculated at ages ranging from 1-3 week post germination. Conidial and mycelial suspension of the pathogen were prepared from 8 days old culture grown on TeDA medium. Aqueous conidial suspension of the pathogen was amended with surfactant (.02% Tween-80) and standardized at 2×10^5 spores/ml with a hemocytometer. Plants were injured with sterilized needle and inoculated by spraying the leaf and stem surfaces to run off with the conidial suspension. Controls were sprayed with sterilized distilled water plus surfactant only. Plants were monitored daily for 2 weeks for studying the development of disease.

Results and Discussion

During the extensive surveys conducted by the authors in the states of Haryana and Punjab in the years 1989 – 1998, infestation of horse purslane was recorded in mustard, maize, arhar, soybean, potato and onion crops. Up to 60-70% infestation of this weed was reported in arhar and soybean fields and 80-90% in maize and mustard. Several workers have earlier reported heavy infestation of *Trianthema* in black gram (Mohamed Ali and Durai, 1987), cotton (Tiwana and Brar, 1990; Brar *et al.*, 1995), onion (Singh *et al.*, 1992), pearl millet (Rathee *et al.*, 1992; Balyan *et al.*, 1993), pigeon pea (Chauhan *et al.*, 1995) and sugarcane (Phogat *et al.*, 1990). The weed reduced mung bean yield by 50-60% when left

untreated (Balyan, 1985; Balyan and Malik, 1989) and pearl millet yield 80-89% (Balyan, 1985).

In August 1994, a mustard field, found heavily infested with horse purslane and in an advanced state of decline with dead leaves was noticed. Leaf spots and necrosis were the common symptoms observed. Although all the stages of leaves showed infection, the mature leaves were more heavily affected. Initially the lesions on the leaves were round pinpoints, which later on became round to irregular, necrotic, straw colored with maroon margins. Lesions often coalesced and leaves abscised resulting in premature defoliation.

In moist chambers, growth of the fungus with conidiophores and conidia was observed on infected portions, 3 days after incubation. Isolation from diseased horse purslane leaves on PDA plates yielded *Gibbago trianthemae* Simmons, whose identity was confirmed by CAB International Mycological Institute, England (IMI Nos. 366407, 377851).

Typical disease symptoms were produced on both injured and uninjured leaves *in vitro* and *in vivo* and the inoculated pathogen was reisolated and found similar to the original isolate in cultural characteristics thus confirming the pathogenicity of *G. trianthemae* to *Trianthema portulacastrum* and competing the Koch's postulates.

Gibbago trianthemae on *T. portulacastrum* is a new record for India (Aneja and Kaushal, 1999) and second for the world. Earlier it was described from the USA, Cuba and Venezuela as a new phaeodictyoconidial genus of Hyphomycetes (Simmons, 1986).

In the present study best growth was found on TeDA, followed by PDAY > PDA media. Growth was good on PSA > V-6 juice agar > NA > ME and very poor growth was noticed on CDAY > CDA and MA media. Sporulation was best on TeDA, PDAY and PDA ($8.6 \times 10^5 > 8.0 \times 10^5 > 7.37 \times 10^5$ conidia/ml respectively). *Gibbago* also sporulated well on NA, ME and V-6 juice agar media ($5.3 \times 10^5 > 5.30 \times 10^5 > 4.78 \times 10^5$ conidia/ml). Poor sporulation was observed on CDAY, PSA, CDA and MA media ($3.12 \times 10^5 > 3.05 \times 10^5 > 2.2 \times 10^5 > 4.14 \times 10^4$ conidia/ml). Both growth and sporulation of the pathogen were found to be better on TeDA than all other media tested, thus suggesting that the trianthema extract can be used as a good substitute for culturing this fungus.

Stained sections of leaves observed 2-3 days after inoculation revealed that germ tube enters into the host cell by the formation of appressoria. Germ tubes within the cortical cells showed the formation of intermediate swelling from which infection hyphae arise which later penetrate into adjacent cells. Hyphae reached the cortical cells within 144 - 168 hours after inoculation. Plant tissue at the infected site collapses and extensive ramification of the hyphae in the host's cortical cells results 8-10 days after spraying of inoculum.

Appearance of symptoms on leaves started after 3-4 days of spraying of inoculum. Initially symptoms were pinpoint, black with maroon margins upto 1 mm in diameter. The lesions became sunken and necrotic after 7-9 days of inoculum spraying. Lesions often coalesced and abscised resulting in premature defoliation. As the lesions elongate they also expanded around the stem. Our observations revealed that it is highly pathogenic to horse purslane as evidenced by the rapid rate of infection and colonization of the host.

In the treated pots, infection appeared on the leaves in the form of small pin point lesions 3-4 days after inoculation, which eventually become enlarged, necrotic and straw coloured 7-10 days post inoculation. Lesions often coalesced and defoliation of leaves started 20 days post inoculation. Upto 72-84% infection was observed on the leaves, artificially inoculated with an inoculum of 2×10^5 conidia/ml 30 days post inoculation. In spite

of the severe infection, new leaves continue to emerge on a few plants and plants survived in a few cases.

Statistical analysis of the data on the inoculated and uninoculated plants, revealed that percent infection of horse purslane leaves was highly significant in inoculated plants in both covered and uncovered pots (all calculated t-ratios exceed the table values with 6 degree of freedom at .01 probability level) (Table 1). Comparison of data in covered and uncovered pots revealed that more infection occurred in uncovered pots as compared to covered pots, but the difference in means was not significant statistically (t value less than table value with 6 degree of freedom). However, the foliar application of the pathogen resulted in the reduction in the production of number of leaves, height and biomass/plant. The reduction in number of leaves was 36-37% in both covered and uncovered pots; reduction in plant height in covered pot was 53% and uncovered pots 60%. The biomass / plant was also reduced to 65% in covered pots and 85% in uncovered pots. The differences in the production of number of leaves, height and biomass per plant were found to be statistically significant (Table 2).

Table 1.
Effect of foliar application of *Gibbago trianthemae* to horse purslane (percent infection)

Treatment	Percent infection on leaves ^a	
	Covered Pots	Uncovered Pots
Inoculated ^b	72.24±7.70 ^{ce}	84.82±4.41 ^{ce}
Control ^d	3.8±0.0	8.55±3.5

a = Each value represents the average of 6 replicates, each replicate contained an average of 5 plants / pot

b = Plants were sprayed to runoff with a suspension of 2.2×10^5 conidia per milliliter+0.2% surfactant

c = The two values within columns are significantly different as determined by the t-test

d = Plants were sprayed to runoff with surfactant and water only.

e = The two values followed by letter e are not significantly different as determined by t test.

Table 2.
Effect of foliar application of *Gibbago trianthemae* to horse purslane (leaves, height and biomass), 30 days after inoculation

Treatment	Average no. of leaves/plant ^a		Average height/plant ^a		Average biomass/plant ^a	
	Covered	Uncovered	Covered	Uncovered	Covered	Uncovered
Inoculated	22.6±1.70 ^{bc}	23.4±1.52 ^{bc}	10.62±0.65 ^{bc}	13.2±0.71 ^{bc}	0.26±0.07 ^{bc}	0.36±0.4 ^{bc}
Control	35.4±1.36	38.2±1.58	23.19±3.18	29.85±3.15	16.2±0.13	0.98±1.7

^a Average of six replicates

^b The value within columns are significantly different as determined by t-test ($p \geq 0.01$)

^c The two values followed by letter c are not significantly different as determined by t-test ($p \geq 0.05$)

Host range studies conducted on 12 plant species (3 weeds and 9 agricultural crops) it revealed that none of these except one i.e horse purslane showed symptoms of the disease (Table 3).

Table 3.
Response of various crop/weed species tested for susceptibility to
Gibbago trianthemae

S. No.	Host	Family	Disease Reaction
Weeds			
1	<i>Trianthema portulacastrum</i>	Aizoaceae	S
2	<i>Amaranthus viridis</i>	Amaranthaceae	R
3	<i>Chenopodium album</i>	Chenopodiaceae	R
Crops			
4	<i>Triticum aestivum</i>	Poaceae	R
5	<i>Zea mays</i>	"	R
6	<i>Sorghum vulgare</i>	"	R
7	<i>Oryza sativa</i>	"	R
	<i>Avena sativa</i>	"	R
9	<i>Hordeum vulgare</i>	"	R
10	<i>Brassica campestris</i>	Cruciferae	R
11	<i>Cajanus cajan</i>	Fabaceae	R
12	<i>Glycine max</i>	"	R

S=Susceptible ; R=Resistant

The present data suggest that *G. trianthemae* can be highly aggressive towards horse purslane and has certain characteristics suggested by various workers (Aneja, 1999) that make it a desirable candidate as biological control agent of a weed, such as: capable of limiting population without eliminating the species; can be easily cultured on natural host; good sporulation capacity; narrow host range, fast growth rate and hence can be mass produced in a short time and should not be ruled out as a possible agent with biocontrol potential of this weed in India. Intensive work is still needed on the impact of the field environment and application technology on the efficacy of this pathogen as a mycoherbicide.

Acknowledgements

The authors are thankful to the Director, CAB International Mycological Institute, Egham, England for confirming the identification of the isolate.

References

- Aneja, K.R. 1999. Biotechnology for the production and enhancement of mycoherbicide potential. In From Ethnomycology to Fungal Biotechnology, J. Singh and K.R. Aneja [eds.]. Kluwer Academic/Plenum Publishers, Netherlands/UK. pp. 91-114.
- Aneja, K.R., and S. Kaushal. 1999. Occurrence of *Gibbago trianthemae* on horse purslane in India. J. Biol. Control. 12 (2) : 157-159.
- Aneja, K.R., and K. Singh. 1989. *Alternaria alternata* (Fr.) Keissler, a pathogen of water

- hyacinth with biocontrol potential. *Trop. Pest Manag.* 35 (4): 354-355.
- Balyan, R.S. 1985.** Studies on the biology and competitive behaviour of carpetweed (*Trianthema portulacastrum* L.). Ph.D. Thesis, HAU, Hisar.
- Balyan, R.S., and V.M. Bhan. 1986.** Emergence, growth and reproduction of horse purslane (*Trianthema portulacastrum*) as influenced by environmental conditions. *Weed Sci.* 34: 516-519.
- Balyan, R.S., and R.K. Malik. 1989.** Control of horse purslane (*Trianthema portulacastrum*) and barnyardgrass (*Echinochloa crus-gali*) in mung bean (*Vigna radiata*). *Weed Sci.* 37: 695-699.
- Balyan, R.S., S. Kumar, R.K. Malik, and R.S. Panwar. 1993.** Post-emergence efficacy of atrazine in controlling weeds in pearl-millet. *Indian J. Weed Sci.* 25(1 and 2): 7-11.
- Brar, A.S., R.J.S. Thind, and L.S. Brar. 1995.** Integrated weed control in upland cotton (*Gossypium hirsutum* L.). *Indian J. Weed Sci.* 27(3 and 4): 138-143.
- Chauhan, D.R., Balyan, R.S., Kataria, O.P. and Dhankar, R.S. 1995.** Weed management studies in pigeonpea (*Cajanus cajan*). *Indian J. Weed Sci.* 27(1and2): 80-82.
- Duthie, J.F. 1960.** Flora of the Upper Gangetic Plain. Periodical Experts, Delhi. pp.500.
- Mitchell, J.K. 1988.** *Gibbago trianthemae*, a recently described hyphomycete with bioherbicide potential for control of horse purslane (*Trianthema portulacastrum*). *Plant Disease*, 72: 354-355.
- Mohamed Ali, and R. Durai. 1987.** Control of *Trianthema portulacastrum* L. in blackgram. *Indian J. Weed Sci.* 19: 52-56.
- Mortensen, K. 1998.** Biological control of weeds using microorganisms. In *Plant-Microbe Interactions and Biological Control*, G.J. Boland and L.D. Kuykendall [eds.]. Marcel Dekker Inc., New York. pp. 223-248.
- Phogat, B.S., V.M. Bhan, and R.S. Dhawan. 1990.** Studies on the competing ability of sugarcane with weeds. *Indian J. Weed Sci.* 22(1 and 2): 37-41.
- Rathee, S.S., R.K. Malik, and S.S. Punia. 1992.** Effect of time of nitrogen application and weed management on pearl millet. *Indian J. Weed Sci.* 24 (1 and 2): 17-21.
- Schroeder, D., and H. Muller-Scharer. 1995.** Biological control of weeds and its prospectives in Europe. *Med. Fac. Landbouww Univ. Gent.* 60(2a): 117-124.
- Simmons, E.G. 1986.** *Gibbago*, a new phaeodictyoconidial genus of hyphomycetes. *Mycotaxon* 27: 107-111.
- Singh, S.J., K.K. Sinha, S.S. Mishra, S.S. Thakur, and N.K. Choudhry. 1992.** Studies on weed management in onion (*Allium cepa* L.). *Indian J. Weed Sci.* 24(1 and 2): 6-10.
- Tiwana, U.S., and L.S. Brar. 1990.** Effect of herbicides on weed control efficiency and production potential of American cotton (*Gossypium hirsutum* L.) *Indian J. Weed Sci.* 22(3 and 4): 6-10.
- Wapshere, A.J. 1974.** A strategy for evaluating the safety of organisms for biological weed control. *Annals Appl. Biol.* 77: 201-211.