Isolation, identification and pathogenicity assay of fungal species on Horse purslane (*Trianthema portulacastrum L.,*)

ABSTRACT

A field survey was carried out in Tamil Nadu agricultural university, Coimbatore, to locate Horse purslane (*Trianthema portulacastrum)* infected with fungal pathogens. Heavily infested areas were recognized and symptomatic leaves were collected. Fungal species account for leaf spot disease of Horse purslane were Isolated from infected leaves on PDA medium. Based on morphological characteristics two foliar pathogens of Horse purslane were identified as *Gibbago trianthemae* and *Curvularia tuberculata*. Pathogenicity of two isolates was tested by applying spore treatment to Horse purslane (test plants). Leaves of test plants showed naturally occurring symptoms after inoculation with pathogens. Among the two isolates, *Gibbago trianthemae* was shown to be more effective against Horse purslane than *Curvularia tuberculata.*

Keywords: Curvularia tuberculata, Gibbago trianthemae, Horse purslane, identification, isolation, pathogenicity, Trianthema portulacastrum.

1. INTRODUCTION

Horse purslane (*Trianthema portulacastrum*), an annual herb in the family Aizoaceae, is one of the main weeds in agriculture [1].*Trianthema portulacastrum* is also known as Desert horse purslane, Giant pigweed (English), and *Vishakhapara* (Hindi).It was discovered as a noxious weed plant in several agricultural crops in tropical and subtropical areas including India, where it offers competition and reduces crop yields by 30% to 60% [2]. It is an introduced weed in India, however competition for yields in various agricultural and vegetable crops like mustard, maize, pigeon pea, soybean, potato, and onion crops, it has turned into a noxious weed [3, 4]. Due to the great seed production and brief dormancy of Horse purslane, several generations can occur in a single season.when all of these adverse

traits are taken into account, effective control tactics are needed for managing Horse purslane.

Many farmers are switching to organic farming and looking at different weed control options due to concerns about ecological, environmental and health issues associated with the widespread use of herbicides [5, 6]. An effective substitute for synthetic herbicides and a sustainable method of environmental protection would be biological control. Utilizing live organisms like insects, nematodes, bacteria, or fungi can help manage weed populations biologically. It has been revealed that many parasitic, aquatic, woody, climbing, and herbaceous weeds are susceptible to plant pathogens [7].The biological management of weeds using plant pathogens has become increasingly relevant since it is effective, safe, practicable, safe, and environmentally acceptable [8, 9].

Among plant pathogens, the most diverse group is the fungi, which may infect many different types of plants and cause a wide range of illnesses. In recent years, fungal pathogens have been known to cause stem blight and leaf spot diseases in horse purslane. Horse purslane, which ultimately causes complete defoliation and drying of the weed [10].In the present study, with the aim of controlling Horse purslane biologically, isolation and identification of pathogens responsible for the infection of Horse purslane was done and tested for pathogencity.

2. MATERIAL AND METHODS

2.1. Isolation of mycoflora from *Trianthema portulacastrum*

Diseased leaves of *Trianthema portulacastrum* were collected from farms at the Tamil Nadu Agricultural University, Coimbatore. To get rid of dirt and other debris, symptomatic leaves were carefully cleaned under running water. The infected leaf tissue was divided into small fragments of 1 cm-diameter, surface sterilized with ethyl alcohol for 1-2 minutes, and then washed with distilled water. Leaf fragments are soaked for one minute in mercuric chloride solution, and then cleaned with sterile water before being placed in petriplates with potato dextrose media supplemented with streptomycin sulphate, which inhibits bacterial growth. With all necessary precautions taken to prevent contamination, leaf fragments were inoculated in petriplates, under laminar air flow. Petriplates were placed in an incubator after inoculation to create ideal conditions for pathogen growth, specifically i.e. 25±2 under 12hrs dark/light period. Sub culturing was done from 7 days old culture, and allowed pathogen to grow completely.

2.2. Pathogen identification

Microscopic observations were carried out from completely grown culture plate. The

identification features of each isolates such as colony diameter, colour, texture, sporulation,shape and sizes of conidiophores and conidia were carefully studied. Fungal isolates were identified with the help of pertinent literature [11, 12, 13].

2.3. Formulating a mycoherbicidal solution

Potato dextrose broth was prepared and autoclaved at 121°C for 30 minutes. Discs of two pathogens were inoculated in conical flasks of 250 ml and allowed to grow for 15 to 20 days at room temperature. After formation of fungal mat it was removed from flask and crushed in mixerand diluted with water in different quantities for spraying. A wetting agent was added while spraying to ensure that every leaf gets an even coating of moisture.

2.4. Pathogenicity test

During the survey, Horse purslane seeds were collected from agricultural fields. The collected seeds were dried and preserved in healthy condition without any contamination. The seeds were planted in plastic pots filled with sterilized soil to grow the plants. A green house with a 12 hour light/dark photoperiod was used to sustain the pots containing weed seedlings. Prior to the inoculation of the pathogen spores, test plants were handled carefully to prevent pre-infection by other contaminants.

2.4.1. Inoculation of test plants

The assay on the infection process was conducted on the healthy, greenish plants growing in the greenhouse chamber. 12 pots were used for the inoculation of two pathogens with five different concentrations, along with a control. To prevent the spores from drying out, inoculations were carried out by 6 and 7 pm after sunset. The plants were sprayed with formulations of the two pathogens, *Gibbago trianthemae* and *curvularia tuberculata* at concentrations of 2, 4, 6, 8 and 10 with 10^6 spores/ml, as well as a control[8], when the test plants had 10 to 15 leaves. Spore inoculum along with a 0.02% wetting agent is applied to test plants. Control plants received the same treatment except that they were sprayed with sterile water + 0.02% wetting agent. At intervals of 10 days, leaves are checked for symptoms.

The leaves were rated for disease based on the spread of the disease on them, by using the ranking scheme [13].

0- no sign of illness, a healthy plant;
1- mild symptoms, a plant with few symptoms on 1 to 10% of the leaf area;
2- moderate symptoms, a plant showing larger diseased areas on 11 to 25% of the leaf area;

3- less severe symptoms, includes enlarged lesions that cover 26 to 50% of the leaf area;
4- severe, symptoms cover 50 to 75% of the leaf area; and
5- Very severe, more than 75% of leaf area covered with symptoms

Disease intensity / Percent Disease Index were calculated by formula given by [14].

$$\text{Percent Disease Index}=\frac{\text{sum of ratings}}{\text{no of leaves observed}\times\text{highest rating}}\times 100$$

Chart 1: Treatment details

Treatments	Concentration of formulations(cfu/g)
T_1	*Gibbago trianthemae, Curvularia tuberculata* each @2×10^6 spores/ml
T_2	*Gibbago trianthemae, Curvularia tuberculata* each @4×10^6 spores/ml
T_3	*Gibbago trianthemae, Curvularia tuberculata* each @6×10^6 spores/ml
T_4	*Gibbago trianthemae, Curvularia tuberculata* each @8×10^6 spores/ml
T_5	*Gibbago trianthemae, Curvularia tuberculata* each @10×10^6 spores/ml
T_6	control

3. RESULTS AND DISCUSSION

Two pathogens were isolated from infested leaf portion of Horse purslane and by microscopic observations they were identified as *Gibbago trianthemae* and *Curvularia tuberculata*.

3.1. Morphological characters of *Gibbago trianthemae*

Culture colour is a greyish-white with cottony growth [Fig 1]. The mature culture appears black in colour. On PDA, the subsurface mycelia development was black and dense and the fuzzy aerial mycelium appeared with moderate levels of sporulation. Mycelia was pale straw-colored and septate. Conidia produced in culture were characterized by means of secondary conidiophores.Conidiophores simple or 1-2 proliferated. 1-4 transeptate, pale straw- colored.Conidia solitary, almost completely ellipsoid, with 2 entire longitudinal septa intersecting at right angles in each conidium half, 1-4 whole or partial transverse septa, and a few shorter septa in transverse sectors

Fig 1: Colony of *Gibbago trianthemae*

3.2. Morphological characters of *Curvulariatuberculata*

Culture on PDA is brown [Fig 2], mycelium on a natural substrate is typically submerged, and the hyphae are branching, septate, and brown, smooth or verrucose. Stomata generally large, upright, black, cylindrical, sometimes branched. Mycelial colour ranges from grey to black and has septa.Conidia are straight or curved, ovoid, obclavate or ellipsoidal, 3-5 septate, intermediate cells brown to dark brown, end cells sub hyaline to pale or dark brown, mature conidia tuberculate. Young conidia are sub hyaline and smooth**.** First septum in the conidium is usually median, second septum often delimiting the basal cell but variations in septal formation may occur.

Fig 2: Colony of *Curvularia tuberculata*

3.3. Growth of two isolates: On PDA media *Curvularia tuberculata* growth was faster when compared to *Gibbago trianthemae*.

Table 1.Colony growth *of Gibbago trianthema*

Days after sub culturing	colony diameter(cm)
1	0.5
2	4
3	5
4	7

Table 2.Colony growth of *Curvularia tubercualta*

Days after sub culturing	Colony diameter(cm)
1	1
2	5
3	6
4	9

3.4. Assay on the pathogenicity of fungal species

At 10, 20, 30, and 40 days after inoculation (spore treatment) on Horse purslane, the pathogenicity of fungal species was recorded. From each pot of green house plants, a random sample of leaves was taken to figure out the infection rate. The leaves were assessed using a disease rating scale based on the increase in disease area, and a percent disease index was calculated for various treatments. Test plants inoculated with *Gibbago trianthemae* showed more symptoms [Fig 3] and high percent disease index values than *curvularia tuberculata* [6, 7].

Table 3.Disease intensity on test plants inoculated with *Gibbago trianthemae*

S.No	Treatment Details	Percent Disease Index (%)			
		10DAT	20DAT	30DAT	40DAT
1	**T_1-***Gibbago trianthemae*@2× 10^6 cfu/g	0.00 (0.00)	9.77 (18.20)	19.54 (26.22)	28.40 (32.19)
2	**T_2-***Gibbago trianthemae*@4×10^6 cfu/g	0.00 (0.00)	16.02 (23.59)	21.48 (27.57)	34.72 (36.08)
3	**T_3-***Gibbago trianthemae*@6×10^6 cfu/g	0.00 (0.00)	21.21 (27.39)	27.03 (31.31)	43.34 (41.17)
4	**T_4-***Gibbago trianthemae*@8×10^6 cfu/g	7.95 (16.37)	25.96 (30.63)	31.84 (34.35)	50.78 (45.44)
5	**T_4-***Gibbago trianthemae*@8×10^6 cfu/g	12.56 (20.75)	30.30 (33.20)	42.34 (40.59)	68.50 (55.90)
6	**T_6**-control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

SEd	0.39	1.34	1.63	2.28
CD 5%	0.96	3.27	3.99	5.59
CD 1%	1.45	4.96	6.04	8.47

Datainparenthesesare arcsine √per cent angular transformed values
DAT=Days after treatment

Table 4.Disease intensity on test plants inoculated with *curvularia tuberculata*

S.No	Treatment Details	Percent Disease Index (%)			
		10DAT	20DAT	30DAT	40DAT
1	**T_1**-*Curvularia tuberculata* @2×10^6 cfu/g	0.00 (0.00)	0.30 (3.09)	12.39 (20.59)	16.24 (23.74)
2	**T_2**-*Curvularia tuberculata* @4×10^6 cfu/g	0.00 (0.00)	9.06 (17.49)	16.86 (24.23)	21.92 (27.87)
3	**T_3**-*Curvularia tuberculata*@6×10^6 cfu/g	0.00 (0.00)	12.17 (20.42)	20.22 (26.71)	26.57 (31.02)
4	**T_4**-*Curvularia tuberculata*@8×10^6 cfu/g	0.00 (0.00)	13.41 (21.46)	23.50 (28.96)	32.33 (34.64)
5	**T_5**-*Curvularia tuberculata*@10×10^6 cfu/g	0.00 (0.00)	17.29 (24.56)	26.67 (31.09)	41.48 (40.09)
6	**T_6**-control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEd		-	1.00	1.49	1.70
CD 5%		-	2.46	3.66	4.17
CD 1%		-	3.72	5.54	6.32

Data in parentheses are arcsine √per cent angular transformed values
DAT=Days after treatment

Table 5.Pathogenicity of isolates on Horse purslane after spore treatment

Fungal Species	Isolated part	Score	Disease intensity
Gibbago trianthemae	Leaf	4	Severe symptoms
Curvularia tuberculata	Leaf	2	Moderate symptoms

Fig 3: Plants treated with *Gibbago trianthemae*

Fig a

Fig b

Fig c

Leaf with small lesions, Leaf with increased lesions, and completely dried plant

Fig 4: Plants treated with *Curvularia tuberculata*

Fig a

Fig b

Fig c

Leaf with lesions, partially dried plant, and Control plant

4. CONCLUSION

Two fungal species were isolated and identified from diseased leaves of Horse purslane *(Trianthema portulacastrum)* which was major weed in agricultural crops in research area. Pathogenicity test was done by spore treatment on test plants grown in green house conditions and it was revealed that the isolate *Curvularia tuberculata* displayed mild symptoms on the host plant, and has low percent disease index than *Gibbago trianthemae*, which is more pathogenic, quickly infected the host plants. Hence *Gibbago trianthemae* can be used aspotent biocontrol agent against Horse purslane.

REFERENCES

1. Aneja K.R, Kumar V,Sharma C. Leaf-spot disease of Trianthema portulacastrum – a new record from world. Journal of Innovative Biology. 2014; 1(2): 112-116.

2. Balyan, R S, Malik R K. Control of horse purslane (Trianthema portulacastrum) and barnyard grass (Echinochloa crus-gali) in mung bean (Vigna radiata). Weed Science. 1989; 37(5):695-699.

3. Hazra, Dipak, Das T.K, Yaduraju. Interference and economic threshold of horse purslane (Trianthema portulacastrum) in soybean cultivation in northern India. Weed Biology and Management.2011; 11: 72–82.

4. Saeed M,Marwat K B, Hassan G, Khan A. Interference of horse purslane (Trianthema Portulacastrum L.) with maize (Zea Mays L.) at different densities. Pakistan Journal of Botany.2010; 42:173-179.

5. Duke S, Dayan F,Rimando A, Schrader K, Aliotta G, Oliva A ,Romagni J. Chemicals from nature for weed management. Weed Science.2002;50(2): 138-151.

6. Walz, E. Third biennial national organic farmers survey Organic farming research foundation. Santa Cruz, CA. 1999.

7. Charudattan, Raghavan.The Mycoherbicide Approach with Plant Pathogens. Microbial control of weeds. 1991; 24- 57.

8. Charudattan R. Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. Biological Control.2001;46:229-260

9. Bouda H, Tapondiou L A, Fontem D A, Gumedzoe M Y.Effect of essential oils from leaves of Ageratumconyzoides, Lantana camara and Chromolaenaodorata on the mortality of Sitophiluszeamais (Coleoptera). Journal of Stored Products Research .2001; 37: 103-109.
10. Rubén F G, Ana B V L, Rosario A F C , Ignacio E M M. First report of stem blight and leaf spot in horse purslane caused by gibbago trianthemae in sinaloa, mexico, Canadian journal of plant pathology.2021; 43(3): 431-438.

11. Gaddeyya G, Ratna kumar P K, Bharathi P. selection of some fungal pathogens for biological control of trianthema portulacastrum l., a common weed of vegetable crops. Journal of applied biology and biotechnology. 2016; 4 (04): 090-096.

12. Kumar V & Aggarwal N, Aneja K,Kaur M. Gibbago trianthemae , phaeodictyoconidial genus, cause leaf spot disease of Trianthema portulacastrum. Archives of Phytopathology and Plant Protection. 2016; 49: 1-11

13. Ray P Hill MP, Impact of feeding by Neochetina weevils on pathogenicity of fungi associated with waterhyacinth in South Africa. Journal of Aquatic Plant Management 2012; 50: 79–84.

14. Hossain MT, Hossain SMM, Bakr M A, MatiarRahman A K M, Uddin S N. Survey on major diseases of vegetable and fruit crops in Chittagong Region. Bangladesh Journal of Agricultural Research. 2010;35(3): 423-429.

.