Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 305-307
Received: 03-03-2018
Accepted: 07-04-2018

SK Khatik
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

K Mathur
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

GK Bagri
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

R Kumari
Department of Plant Protection, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

DK Bagri
Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

DL Bagdi
Department of Plant Physiology, SKN. College of Agriculture, Jobner, Rajasthan, India

Correspondence
DL Bagdi
Department of Plant Physiology, SKN. College of Agriculture, Jobner, Rajasthan, India

Standardization of methods of mass production and application of *Gibbago trianthemae* on *Trianthema* portulacastrum

SK Khatik, K Mathur, GK Bagri, R Kumari, DK Bagri and DL Bagdi

Abstract
Thirteen different formulation consisting of *G. trianthemae* spores, inert carrier, Tween 20 and oils (groundnut, mustard and Paraffin oil) were evaluated for disease development on pots -grown plants of *T. portulacastrum* in green house. The Maximum per cent disease index (PDI), the least fresh weight and dry weight were found 100 per cent, 2.8 g and 0.56 g respectively with *G. trianthemae* spores + Kaolin + Parafine oil + Water, followed by that having *G. trianthemae* spores bentonite + Parafine oil + Water formulation.

Keywords: *Gibbago trianthemae, Trianthema portulacastrum,* formulations

Introduction
In India, little work has been done on mycoherbicides. Though some pathogens exlusively infecting most problematic weeds have been reported as new records. On *Parthenium hysterophorus* (Congress grass) *Alternaria zinniae* and *Phoma sorghina* (Kumar and Kumar, 2000a) [9]; *Alternaria alternata, Sclerotium* sp. and *Aspergillus niger* (Joshi *et al.* 2001) [8] have been reported while on *Lantana camara Alternaria* spp., *Phoma* spp., *Fusarium* sp. and *Curvularia* sp. have been reported to cause moderate to severe disease (Saxena and Pandey, 2000) [12].

Trianthema partulacastrum L., commonly known as horse purslane, black pigweed, carpet weed, gudbur, hogweed, itcit or santha is an important terrestrial weed of the family Aizoaceae. It is indigenous to South Africa, but now has spread into tropical and sub-tropical countries such as West Asia, India, Sri Lanka, Africa and Tropical America. *T. portulacastrum* has been reported to be very aggressive weed in mustard, maize, pegion pea, soybean, potato and onion crops in the states of Haryana and Punjab. Up to 60-70 per cent infestation of this weed has been reported in pigeon pea and soybean fields. Because of its infestation in various agricultural crops especially during the rainy season, horse purslane has been referred to as number one problematic weed of agricultural crops in Northern India (Singh and Prasad, 1994, Aneja and Kaushal, 1999) [1].

The pathogens reported on *T. porulacastrum* are *Cercospora trianthemae Gibbago Trianthemae*; *Drechslera* indica (=*Bipolaris indica)* Taber *et al.* 1998 [15] and soil born pathogen (*Fusarium oxysporum*); Darshika and Daniel, 1992). Babu *et al.* (2004b) [4] reported a leaf blight of *T. portulacastrum* caused by *Paecilomyces varioti* as a new host record. Bohra *et al.* (2005) [5] evaluated a strain of *Alternaria alternata* as mycoherbicies of *T. protulacastrum,* A strain of *Myrothecium verrucaria* applied of 2×10^7 conidia ml^{-1} also killed Horse purslane seedlings in addition to spotted spurge and prostrate spurge in tomato field (Boyette *et al*., 2007) [6].

Materials and Methods
For standardizing methods of mass production and application of *G. trianthemae*, its formulations using fungal growth on culture media + inert carriers (Kaolin, talc and bentonite) were prepared and evaluated on pot- grown plants of *T. portulacastrum*. Different formulations using *G. trianthemae* spore suspensions as well as its water in oil in water (WOW) formulations using vegetable oils (ground nut and canola) and mineral oil were spray inoculated on *T. portulacastrum* plants.

Seeds of (*Trianthema portulacastrum*) were sown in sterilized soil and earthen pots (15 cm diameter) for each treatment ten plants were used. The experimental design was completely randomized design

For preparation of the inoculum pure culture of *G. trianthemae* was grown for 14-15 day in petri plates on chickpea flour agar. The spores were harvested by flooding the plates with sterile distilled water and gently scrapping the colony with the help of a sterilized plastic loop and the conidial suspension was strained through muslin cloth. The spores of *Gibbago trianthemae* were added separately with kaolin, Talc and bentonite to produce a powder containing 1×10^9 spores g^{-1}.

Liquid formulations containing 1×10^6 spores ml^{-1} were subsequently prepared as follows. For a water based suspension, 0.2g of kaolin/spores powder, Talc/spores powders and Bentanitc/ spores powder each was added to 200 ml water in a 250 ml conical flask. For suspension emulsions (10% oil; 1% emulsifier), 0.2 g of kaoline, Talc and Bentonite formulation powder was mixed with 20 ml of vegetable oil with a magnetic stirrer in a 250 ml conical flask; 2 ml of which Span 80 (*Sorbitan monnooleate*) proved, a satisfactory emulsifier for all the oils was added as an emulsifying agent and mixed water was then added to a total volume of 200 ml while stirring continuously. The vegetable oil used was canola (Rapeseed) peanut (Chetal) paraffin mineral oil.

The various formulations were spray inoculated on 15- days-old plants using an atomizer and spraying to incipient run off. The controls received only water sprays.

The inoculated plants were kept in humid chamber for 24 hours and then transferred to cage house and high humidity was maintained. The disease severity was recorded on 10^{th} day on a standard 1-5 scale. In this scale (1= free from disease, 2= 10 per cent leaf area infected, 3= 11-25 per cent leaf area infected, 4= 26-50 per cent leaf area infected and 5= above 50 per cent area of leaf infected) observations on PDI, fresh weigh and dry weigh of pot grown plant were taken. Numbers of plants in each score were recorded and the PDI in each plot was determined as after disease development the disease severity was recorded on a standard 1-5 scale based on per cent of total leaf area covered with lesions or blighted. In this scale score 1= free from disease, 2= 10 per cent leaf area infected, 3= 11-25 per cent leaf area infected, 4= 26-50 per cent leaf area infected and 5= above 50 per cent area of leaf infected). Number of plants in each score was recorded and computed to get PDI.

$$PDI = \frac{n \times 1 + n \times 2 + n \times 3 + n \times 4 + n \times 5}{N} \times \frac{100}{maxi\ diseas\ score}$$

Where

n = number of plants in each score, 1-5 disease score

N = Total number of plant under observation.

The plants were then carefully uprooted, their roots washed and their fresh weigh and dry weigh of pot grown plant were taken numbers of plants in each score were recorded.

Results

Formulations of *G. trianthemae*, using inert carriers (Kaolin, talc and bentonite)and water in oil in water (WOW) formulations using vegetable oils (ground nut and canola) and mineral oil were evaluated on pot- grown plants of *T. portulacastrum*. For this, seeds of (*T. portulacastrum*) were sown in sterilized soil and earthen pots (15 cm diameter), treatment ten plants per pot and three pots as three replications for each treatment were maintained. The experimental design was completely randomized design (Table-1).

The spores of *G. trianthemae* were added separately with kaolin, Talc and bentonite to produce a powder containing 1×10^9 spores g^{-1}. Liquid formulations containing 1×10^6 spores ml^{-1} were subsequently prepared using rapeseed, peanut or paraffin oil. The various formulations were spray inoculated on 10- days- old plants using an atomizer and spraying to incipient run off. The controls received only water sprays.

The inoculated plants were kept in humid chamber for 24 hours and then transferred to cage house and high humidity was maintained throughout the disease development period by frequent irrigations. After disease development the disease severity was recorded on a standard 1-5 scale. Number of plants in each score was recorded and computed to get PDI. Fresh and dry weight of plants was also determined.

All the tested formulations showed significantly higher disease over the *G. trianthemae* spores suspension in water + paraffin oil, as well as reduced fresh and dry weight of the weed. The formulations containing *G. trianthemae* spores + kaolin + parafine oil + water) and bentonite in place of kaolin were the most effective and resulted in 100 per cent PDI and fresh weight and dry weight were, 2.8 g and 0.56 g respectively. *G. trianthemae* formulation with Talc + mustard oil + water resulted in 99.52 per cent disease, 2.9 g, fresh weight and 0.58 g dry weight (Table-1). This was followed by the formulation having Bentonite + Groundnut oil + water, where, PDI, fresh weight and dry weight were 99.13 per cent, 3.0 g and 0.60 g, respectively (Table-1, Plate-1, Fig.-3). In the talc formulation with Groundnut oil + water, PDI, fresh weight and dry weight were 98.09, 3.2 g and 0.64 g respectively, while with Kaolin + Groundnut oil + water (Table-1), PDI, fresh weight and dry weight were 98.02 per cent, 3.3 g and 0.66 g respectively (Table-1). Formulation having Talc + Parafine oil + water resulted in 96.67 per cent disease, 3.4 g fresh weight and 0.68 g dry weight (Table-1), while that with Bentonite + mustard oil + water, had 95.73 PDI, 3.5 g fresh weight and 0.70 g dry weight (Table-1). In formulation having *G. trianthemae* (without any inert carrier), parafine oil + water, PDI, fresh weight and dry weight were 93.76 per cent, 3.7 g and 0.74 g respectively (Table-1), while similar formulation having mustard oil showed 89.50 PDI, 3.8 g fresh weight and 0.76 g dry weight of plants (Table-1). This was followed by the formulation having Kaolin + Mustard oil + water, with 86.09 per cent PDI, 3.9 g fresh weight and 0.78 g dry weight (Table-1). *G. trianthemae* spores suspension in water + Tween 20 resulted in 82.85 PDI, 4.0 g fresh weight and 0.80 g dry weight (Table-1). In the uninoculated untreated pots, PDI was 25.75 per cent, while fresh and dry weights were 46.0 g and 9.2 g respectively (Table-1).

Table 1: Effect of different formulation of *Gibbago trianthemae* on development of disease in *Trianthema portulacastrum* in pots grown plants.

S. No.	Formulation	PDI*	Fresh wt (g) *	Dry wt (g) *
1.	*Gibbago trianthemae* spores + Kaolin + parafine oil + water	100(90)	2.8	0.56
2.	*Gibbago trianthemae* spores + Bentonite + parafine oil + water	100(90)	2.8	0.56
3.	*Gibbago trianthemae* spores + Talc + mustard oil + water	99.52(86.02)	2.9	0.58
4.	*Gibbago trianthemae* spores + Bentonite + groundnut oil + water	99.13(84.64)	3.0	0.60
5.	*Gibbago trianthemae* spores + Talc + groundnut oil + water	98.09(82.05)	3.2	0.64
6.	*Gibbago trianthemae* spores + Kaolin + Groundnut oil + water	98.02(81.91)	3.3	0.66

7.	*Gibbago trianthemae* spores + Talc + parafin oil + water	96.67(79.48)	3.4	0.68
8.	*Gibbago trianthemae* spores + Bentonite + mustard oil + water	95.73(78.07)	3.5	0.70
9.	*Gibbago trianthemae* spores suspension in water in oil in water (WOW) formulation	93.76(75.53)	3.7	0.74
10.	*Gibbago trianthemae* spores suspension in water + mustard oil + Tween 20 ($2ml^{-1}$)	89.50(71.10)	3.8	0.76
11.	*Gibbago trianthemae* spores + Kaolin + mustard oil + water	86.09(68.10)	3.9	0.78
12.	*Gibbago trianthemae* spores suspension in water + Tween 20 ($2ml^{-1}$)	82.85(65.53)	4.0	0.80
13.	Untreated Control (water spray only)	25.75(30.49)	46.0	9.2
	SEm±	3.59	0.20	0.04
	CD at 5%	10.42	0.57	0.13
	CD at 1%	14.09	0.78	0.17
	CV	8.09	5.13	5.75

* Average of three replication; Figures in parentheses are arcsine √ per cent angular transformed values.

Discussion

Most foliar pathogens require several hours of free moisture on plant surface for spore germination, infection structure formation and plant infection. Proper formulation and application technology for any potential mycoherbicide candidate may help in overcoming some of the environmental or biological limitations. Most of the formulations of mycoherbicides involve blending of the fungal spores with inert carriers (diluents or surfactants) in order to alter its physical characteristics to a more desirable and handlable form. Use of anti-desiccants like oils, glycerol, along with surfactants like Tween 20, and inert carriers like bentonite, kaoline, talc, alginate pellets or pesta like formulations of various mycoherbicides have been evaluated by different workers (Egley and Boyette 1995) [7]. In the present study, formulations of *G. trianthemae*, using inert carriers (Kaolin, talc and bentonite) and oils (ground nut, canola and mineral oil) were evaluated on pot- grown plants of *T. portulacastrum*. All the tested formulations showed significantly higher disease over the *Gibbago trianthemae* spores suspension in water + paraffin oil, as well as uninoculated control, and also reduced fresh weight of the weed. The formulation containing *G. trianthemae* spores + kaoline + parafine oil + water) and that having bentonite in place of kaoline were the most effective and resulted in 100 per cent PDI, that was significantly higher compared to *G. trianthemae* spores suspension in water + Tween 20 that resulted in 82.85 PDI. The higher disease incidence also resulted in reduced fresh and dry plant weight. Water –in –oil in Water formulations are reported to be effective as these reduce dew or free moisture dependency of the mycoherbicied The use of vegetable oils, particularly canola oil was found to reduce the dew dependency of the mycoherbicides *Colletotrichum orbiculare* on the weed host *Xanthium spinosum* (Auld, 1993) [2]. Multiple emulsions of water-in-oil-in-water (WOW) has been found to further enhance the field performance of this mycoherbicide (Auld *et al*., 2003) [3]. Similar results for various mycoherbicides have also been reported by several workers (Shivas, 1994; Lawrie *et. al.* 1999 and O'Neill *et. al.* 2000) [11]. Tween 20 (1% V/V) as an adjuvant has also been reported effective for formulation of bioherbicide *Pleospora papaveracea* on *opium* poppy and was found effective in the present studies with *G. trianthemae* also.

References

1. Aneja KR, Kaushal S. Occurrence of Gibbago trianthemae on horse purslane in India. Indian Journal of Biological Control. 1999; 12(2):157-159.
2. Auld BA. Vegetable oil suspension emulsions reduce dew dependence of a mycoherbicide. Crop Protection. 1993; 12(6):477-479.
3. Auld BA, Hetherington SD, Smith HE. Advances in bioherbicides formulation. Weed Biology and Management. 2003; 3:61-67.
4. Babu RM, Sajeena A, Seetharaman K. Paecilomyces varioti as bioherbicide on *Trianthema portulacastrum* (L). A new host record. Journal of Mycology and Plant Pathology. 2004b; 34(1)142-143.
5. Bohra B, Vyas BN, Godrej NB, Mistry KB. Evaluation of Alternaria alternata (Fr.) Keissler for biological control of Trianthema portulacastrum. Indian Phytopathology.2005; 58(2):184- 188.
6. Boyette CD, Hoagland RE, Abbas HK. Evaluation of the bioherbicide Myrothecium verrucaria for weed control in tomato (*Lycopersicon esculentum*). Bio Control Science and Technology. 2007; 17:171-178.
7. Egley GH, Boyette CD. Water-Corn oil emulsion enhances conidia germination and mycoherbicidal activity of Colletotrichum trncatum. Weed Science. 1975; 43(2):312-317.
8. Joshi N, Shenhmer M, Brar KS. Fungal isolates of carrot weed, Parthenium hysterophorus L. from Punjab. Journal of Biological control. 2001; 15:101-102.
9. Kumar PS, Kumar PR. A new leaf spot disease of Parthenium hysterophorus caused by Phoma sorghina. Indian Phytopathology. 2000a; 53:115.
10. Lawrie J, Greaves MP, Down VM. Trematophoma lignicola (Petrak): A potential microbial herbicide for control of Amaranthus retroflexus (L.). BioControl Science and Technology. 1999; 9(3):391-394.
11. O'Neill NR, Jennings JC, Bailey BA, Farr DF. Dendryphion penicillatum and Pleospora papaveracea, destructive seed borne pathogens and potential mycoh erbicides for Papaver somniferum. Phytopathology.2000; 90 (7): 691-698.
12. Saxena S, Pandey AK. Preliminary evaluation of fungal metabolites as natural herbicides for the management of Lantana camara. Indian Phytopathology. 2000; 53:490-491.
13. Shivas RG. Controlled environment studies on the infection of Emex australis by phomopsis emicis. Plant Pathology.1994; 43(3):547-553.
14. Singh G, Prasad R. Studied on the control of *Trianthema portulacastrum* L. in fodder maize. Indian Journal weed Science. 1994; 26:64-67.