SUMMARY

A weed is a plant growing where man wishes other plants or no plants to grow and which has some economic, ecological, or aesthetic implication for man/or his activities. It has been estimated that one tenth of the 300,000 plant species of world are weeds, out of which 300 species cause severe reduction in crop yield and quality. Although herbicides provided effective and reliable control of weeds in crop production systems, but costs, availability, and concerns associated with widespread and increased use bring into question the continued reliance on chemical herbicides. Applicator safety, environmental contamination and population shifts towards more noxious troublesome weeds are some of the concerns which led to the need of cost effective and environmentally acceptable weed control strategies including biological control agents. Biological weed control offers an environmentally benign strategy, because this method has an excellent track record for safety, economically cheaper, free of pollution hazards, and in some cases more efficacious than chemicals. Keeping in view the possibilities that biological agents specially fungal plant pathogens could compete with chemical herbicides or serve as an alternative to chemical control in near future, the present study was undertaken.

In the extensive surveys made in Kurukshetra and its nearby localities, *Trianthema portulacastrum* was found as the most dominant weed infesting all over the areas in wastelands, roadsides, railway tracks, noncultivated area, grasslands and several agricultural crops. In case of agricultural crops, survey showed that *T. portulacastrum* was reported to be a very aggressive weed in mustard, maize, arhar, soybean, onion and potato crops. Upto 60-70% infestation of this weed was reported in arhar and soybean fields and 80-90% in maize and pearl millets fields. In Haryana, survey of agricultural crops revealed that horse purslane was found to be one of the most dominating terrestrial weed among various important crops throughout the state.

During surveys conducted between 2011-14 to search for naturally occurring fungal pathogens on *T. portulacastrum*, a total of six fungal pathogens were identified on the basis of their morphological and microscopically characteristics belonging to four different genera. The conidial shape, structure, morphology and
arrangement of conidia allowed us to identify fungal isolates up to genera level. The extent of damage caused by the fungi to the weed in field situations, together with some other details, viz. taxonomy, previous records, area of damage, cultural characteristics and preliminary biocontrol potential of a few fungi were recorded. Out of six isolated fungal pathogens, four fungal pathogens namely *Cochliobolus australiensis*, *Cochliobolus spicifer*, *Colletotrichum capsici* and *Fusarium chlamydosporum* were isolated first time from *T. portulacastrum*. *Colletotrichum gloeosporioides* and *Gibbago trianthemae* were isolated second time from this weed. Identification of four fungal pathogens was confirmed by Commonwealth Agricultural Bearux International (CABI), International Mycological Institute (IMI), Egham, UK. Molecular sequencing of four fungal pathogens has been done by Macrogen Inc., Advancing through Genomics, Korea. The gene sequences of the fungal pathogens were aligned and have been submitted to National Center for Biotechnology Information (NCBI).

Fungal pathogens were isolated on Potato Dextrose Agar plus Yeast Extract (PDAY) and pathogenicity tests of various fungal pathogens were determined on healthy detached leaves and Koch's postulates were proved. Out of six, five fungal pathogens produce leaf spot symptoms except *Colletotrichum capsici* which produce anthracnose. The symptoms produced by these fungal pathogens and cultural characteristics of these pathogens have been described.

Among fungal pathogens, *Gibbago trianthemae* was considered suitable for further experimentation, because it has been suggested as a probable candidate to control horse purslane weed due to high occurrence frequency, disease intensity, pathogenicity and highly specificity toward host. Among all the ten media tested for growth and sporulation, *G. trianthemae* showed growth on all the ten media, with various growth rates. This fungus showed best growth on PDAY, followed by TEDA > PDA media. Growth was good on PSA > MEA > SDA> NA > V-6 Juice agar and very poor growth was noticed on MA and CDA media. Sporulation was best on TEDA, PDAY and PDA (35.68 x 10^5 > 34.54 x 10^5 > 30.24 x 10^5 conidia/ml respectively). *Gibbago* also sporulated well on PSA, MEA and SDA media (29.31 x 10^5 > 27.41 x 10^5 > 23.57 x 10^5 conidia/ml). Poor sporulation was observed on NA, V-6 juice agar and MA (17.26 x 10^5 > 15.17 x 10^5 > 12.44 x 10^5 conidia/ml). Sporulation was absent in
CDA media. 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.

Twenty three isolates (collected from different regions) of *G. trianthemae* were screened for their best growth and sporulation on PDA+Y and TEDA media and broth at 25°C for 7 days. Out of these 23 isolates, SHB-2-i showed best growth and sporulation on both the media and were used for further studies. In moist chambers, growth of a fungus with conidiophores and conidia was observed on infected portions, after 3-4 days of incubation. Conidia observed were yellow-brown, beakless, muriform, ellipsoid, smooth walled with 3-6 complete or partial transverse septa with constrictions and 1-6 complete or partial longitudinal septa. Among the 11 economically important plants *viz*. *Amaranthus viridis*, *Avena sativa*, *Brassica campestris*, *Cajanus cajan*, *Chenopodium album*, *Glycine max*, *Hordeum vulgare*, *Oryza sativa*, *Sorghum vulgare*, *Triticum vulgare* and *Zea mays* tested for host range studies of *G. trianthemae*, all plants were nonsusceptible to the pathogen, although typical symptoms developed on *Trianthema portulacastrum*, thus showing is host specificity to *T. portulacastrum*.

During studies on the effect of temperature and relative humidity on sporulation of selected fungal pathogen, it was found that the selected pathogen sporulated best at 25°C as compared to all other incubated temp. (*viz.* 5, 15, 45 and 55°C). Relative humidity showed a remarkable effect of the sporulation of selected pathogen. Optimization results showed that best growth and sporulation were observed at 25°C, 5.5 pH, 93% (Relative humidity) for the incubation period of 7 days on PDA+Y medium.

During studies of phytotoxin production, the results of detached leaf and shoot cut bioassay revealed the presence of toxic metabolite in cell free culture filtrate (CFCF) of selected pathogen which reduced the quantity of chlorophyll in both leaves and shoots. Cell free extract of the fungus showed necrosis when injected on leaves of *Trianthema* in moisture chambers. Our results revealed that the CFCF obtained from 21 days old fungal culture was more virulent in comparison of 7 and 14 days old. Further, GCMS evaluation of CFCF showed presence of ten different
compounds as major constituents. Out of these, 3,4-Dimethyl-5-hexen-3-ol showed highest probability and area percentage. In simple laboratory tests highest germination of *Gibbago trianthemae* conidia was achieved when incubated at 25°C ± 2°C, the germination was much reduced at 15 and 35°C and no germination was observed at 5, 45 and 55 °C. Relative humidity showed a remarkable effect on the germination of conidia. Maximum conidial germination was achieved at 100% relative humidity. Germination was very low at 50 % relative humidity. Mycelial discs of the fungus showed growth on agar strips in moist chambers having R.H., 93-100%. No growth of the mycelial discs was observed in moist chambers of R.H. ranging from 52-75%. In controlled environmental conditions, development of typical disease symptoms by *G. trianthemae* on *T. portulacastrum* was most rapid at 25°C. On the basis of our observations, the optimum environmental conditions for growth and infection of host by the pathogen are 25 °C temperature and 90-100% R.H. This gives us an idea that best results of biological potential could be achieved if the inoculum is sprayed at the above temperature and R.H.

Histopathological relationship of *G. trianthemae* with its host, *T. portulacastrum* was investigated by microscopic examination of KOH treated infected leaves collected at different intervals of time. Inoculation of *T. portulacastrum* seedling with suspension of *G. trianthemae* resulted in the formation of small pinpoint lesions on leaves (upto 1 mm diameter) 72 hrs after inoculation. Lesions found within 72-96 hrs after inoculation resulted from penetration of the host through stomata. Conidia germinated after 6-12 hrs after inoculation and entered into host cell. Mycelium grew within host cells and extensive ramification of hyphae was observed within host’s cortical cell, 8-10 days post inoculation, when lesions become enlarged, sunken and got coalesced, resulting in defoliation. On the basis of our observations, the fungus has been found to be highly pathogenic to horse purslane as evidenced by the rapid rate of infection and colonisation of the host by the fungus. In our finding, it was observed that *G. trianthemae* showed cellulose degrading activity in Carboxymethyl Cellulase activity assay (CMCase). Cellulase produced by fungal pathogen may help the pathogen to penetrate into leaf tissue and proliferate inside the leaf.
In the experiments conducted to see the biocontrol potential of *G. trianthemae* on *T. portulacastrum*, pathogen produced typical disease symptoms on the inoculated plants in the experimental pots. Disease, on the leaves, initiated as small pin point lesions, 3-4 days after spraying of inoculum. Lesions later enlarged, become necrotic and straw coloured, 7-10 days after inoculation. Percent infection recorded was 68% in covered pots and 75% in uncovered pots on the leaves, artificially inoculated with an inoculum of $2.2 \times 10^5$ conidia/ml, 30 days after inoculum. The percent infection was highly significant in inoculated and uninoculated plants in (both covered and uncovered pots). Comparison of data in covered and uncovered pots revealed that more infection occurred in uncovered pots, but the difference in means was not significant statically. Application of inoculum gave consistently reduction in the categories of number of leaves, height and biomass per plant. Compared with control treatment, application of inoculum gave 33-54% reduction in the number of leaves in both covered and uncovered pots, 44% reduction in plant height in covered pots and 61% reduction in uncovered pots. Similarly biomass/plant was reduced to 54% in covered pots and 66% in uncovered pots for the same parameter. Difference in the number of leaves, height and biomass per plant in control and inoculated pots were proved significant as determined by t-test.

An adjuvant is any substance in herbicide formation or added to the spray tank to improve herbicidal activity or application characteristics. During studies on the effect of two adjuvants, our results revealed that a total of 10-15% significantly increase in biocontrol potential of selected fungal pathogen when applied with sodium alginate in the conc. of $2.2 \times 10^5$ conidia/ml in pots. It was observed that there was increase in biocontrol potential of pathogen when applied the pathogen with tween 80 but no significantly as compared to sodium alginate.

The phyllosphere/phylloplane study revealed that horsepurslane leaves harboured both bacteria and fungi on their surfaces throughout their life. A total of 10 fungal forms *viz. Drechslera* sp., *Colletotrichum* sp., *Fusarium* sp., *Alternaria* sp., *Curvularia* sp., *Choanephora* sp., *Scopulariopsis* sp., *Nigrospora* sp., *Aspergillus* sp. and *Penicillium* sp. have been isolated by both the dilution plate and leaf impression methods. Bacterial populations outnumbered the fungal population in each sampling. There was a considerable increase in the microbial population with the
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advance in plant age and maturity. The maximum population was obtained at plant maturity stage. A statistical analysis of data revealed that dilution plate method yielded significantly higher bacterial and fungal populations as compared to leaf impression method and dilution plate method was found superior to leaf impression method in our case. None of the phyllosphere fungal isolate was found to be antagonistic to *Gibbago trianthemae*.

Our study concluded that *Gibbago trianthemae* has the characteristics of a potential mycoherbicide such as easy culturability, good sporulation capacity, highly pathogenic to host, narrow host range and good potential to control this weed in the experimental pots. The present data suggest that *G. trianthemae* could be highly aggressive towards horse purslane and has certain characteristics that make it a desirable candidate as biological control agent of this weed.