Asian J. Exp. Sci., Vol. 26, No. 2, 2012; 19-24

Evaluation of Soil Microfauna under *Parthenium hysterophorus* (L.) Vegetation

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Abstract : Experiments were performed to evaluate the microfauna of soil under *Parthenium hysterophorus* vegetation. Soil samples were collected from a depth of 10-15cm, at an interval of 15 days for two months from dense vegetation of *Parthenium* and nearby normal vegetation from agro-ecosystem. Three types of bacterial colony viz. circular-entire, irregular-undulate and punctiform-entire were observed in normal soil while in *Parthenium* affected soil one additional filamentous type of colony was found. All types of colonies showed white pigmentation and Gram positive reaction. Quantitatively the bacterial population of affected soil sample was always significantly less than ($p < 0.001$) normal soil sample. Mean bacterial population of normal soil sample was $33.4\pm0.989\times10^9$, $52\pm0.866\times10^9$, $22.5\pm1.50\times10^9$ and $42.1\pm1.724\times10^9$ and in *Parthenium* affected soil it was $28.3\pm1.921\times10^9$, $49.4\pm1.40\times10^9$, $16.4\pm2.138\times10^9$ and $34.6\pm1.040\times10^9$. A similar trend of significant decline in wet weight (mg /g soil) and biomass (mg /g soil) of bacteria was observed between normal soil samples and affected one. The results indicated that *Parthenium hysterophorus* imparts adverse effect on soil bacteria. The Gram positive bacterial colony with punctiform-entire, shape and margin appeared to be resistant to *Parthenium* infestation in comparisons to other bacteria. Hence, bacteria colony having such type of colony morphology can be used as bioindicator for pollution caused by *Parthenium* .

Keywords : *Parthenium hysterophorus*, Microfauna, Parthenin, Bioindicator.

Introduction

Soil is the reservoir on which most of the life forms on earth depends, as the primary source of food, feed, forage, fiber and pharmaceuticals. Top soil is biologically the most diverse part of the earth and it plays a vital role in sustaining human welfare and assuring future agricultural productivity and environmental stability. An important factor in influencing the productivity of our planet is the nature of their soils. Parr *et al*. (1992) reviewed the different chemical, physical and biological properties of soils that interact to determine the fitness or capacity to produce healthy nutritious crops. It forms a dynamic and complex living system, comprise air, water mineral particles, dead organic matter and various types of living organisms. True soils are influenced, modified and supplemented by living organisms *viz*. micro, meso and macrofauna of soil. Soil microbes, invertebrates and minerals live and work together in an underground system that helps to improve soil and water quality and also to provide greater resistance of plants to pests and disease (Higa, 1994). Soil microorganisms are responsible for decomposition of residual agrochemicals in soil (Higa, 1993), greater mineralization of carbon (Daly and stewart, 1999), more efficient release of nutrients from organic matter (Sangakkara and Weerasekera, 2001) and improved resistant to adverse weather (Higa, 1993) etc. Hence any disturbance in soil microbial population results into adverse effect on the above process which ultimately alters the soil quality.

Quantitative and qualitative microbial activities are the key factors for productivity and sustainability of soils health for maintenance of crop production (Pankhurst *et al*., 1996; Nannipieri *et al*., 2003; Tilak *et al*., 2005). Analysis of structural and functional microbial diversity would, therefore, reveal their sustainability of both man powered and natural ecosystems. Apart from insecticides, pesticides and other various chemicals weed like *Parthenium hysterophorus* (L.) (Heliantheae : Asteraceae; 2n = 34), which is an exotic species of commonly found weed, adversely affect the soil subsystem.

Parthenium hysterophorus has been reported to affect earthworm population (Raipat, 2010), soil bacterial population under experimental condition (Saha *et al*., 2010), and human affairs (Towers and Subba Rao, 1992) etc. but its impact on soil microfauna under natural condition has not been studied.

Although microbial diversity of rice field soil has been nominally investigated (Das and Dangar, 2007a,

b), microbial population of habitats like cropland, botanical gardens, *Parthenium* infested cropland and fallow lands etc. remained unattained to date. The paper deals with the evaluation of soil bacteria (microfauna) under *Parthenium hysterophorus* vegetation to assess the impact of *Parthenium hysterophorus* (L.) on bacterial population.

Materials and Methods

The soil samples were collected with the help of sterilized spatula from a depth of 10-15 cm, at a interval of 15 days for two months from two fields, one having dense vegetation of *Parthenium* and other having normal vegetation. Soil sample of normal soil was labeled as sample A and soil sample collected from the area having dense vegetation of *Parthenium* was labeled as sample B. Enumeration of bacterial population of both the soil samples was done by pour plate technique or dilution plate method on nutrient agar medium (Thornton, 1922; Thom and Raper, 1945). 1 g of both the soil samples was taken and each was dissolved in 9 mL of autoclaved distilled water. Dilutions of 10^{-7} were prepared and 1 mL inoculums of the primary suspension were taken for pouring. Czapek Dox agar media (peptone - 10 g/L, NaCl - 5 g/L, beef extract – 10 g/L, agar – 15 g/L at pH - 7) was used for culture. The Petriplates (diameter 100 mm) were incubated at 37°C for 48 h. For each experiment, three replicates of Petriplates were incubated.

For qualitative analysis, colony morphology (shape-margin), elevation and pigmentation were studied. Quantitative analysis was done by counting number of bacterial colonies (cfu) by colony counter and the results were expressed as 1 g of soil (Table 2). Gram staining reaction of bacterial colony, wet weight and biomass of bacterial colonies of both the soil samples were also analyzed. The mean fresh weight of a bacterium cell was taken as 1.5×10^{-12}g (Toth and Hammer, 1977). This value, when multiplied with the number of bacterial colony, gave the fresh weight of bacteria. Assuming 80% of bacterial cell to be water (Clark and Paul, 1970) biomass of bacterial colony was calculated (Satpathy *et al.,* 1982). T-test was done to determine the significance of change in population and biomass of bacterial colony of both the soil samples.

Results

Qualitative analysis involved morphological details of different bacterial colonies grown on nutrient agar plates (Table 1). The developed bacterial colonies of normal soil (sample A) from shape and margin view point were of three types viz. circular-entire, irregular-undulate and punctiform-entire, while in *Parthenium* affected soil (sample B) one additional type of colony was observed i.e. filamentous. In sample A, 25% of the colonies were circular-entire with 50% flat and 50% convex elevation, 25% colonies were of irregular-undulate type with 50% flat and 50% raised elevation and remaining 50% of the colonies were of punctiform-entire type with flat elevation. All types of colonies showed white pigmentation and gram positive reaction. In sample B, 5% of the colonies were circular-entire, 20% were irregular-undulate, 70% were punctiform-entire and 5% of the colonies were of filamentous type.

Table 1: Morphological details of bacterial colonies in culture condition

Samples	Shape	Margin	Elevation	Pigmentation	Gram reaction
A	25% circular	25% entire	50% flat 50% convex	100% white	Positive
	25% irregular	25% undulate	50% flat 50% raised	100% white	positive
	50% punctiform	50% entire	100% flat	100% white	positive
B	5% circular	5% entire	80% flat 20% convex	100% white	Positive
	20% irregular	20% undulate	50% flat 50% raised	100% white	positive
	70% punctiform	70% entire	100% flat	100% white	positive
	5% filamentous		100% flat	100% white	positive

The elevation of circular-entire colonies were either flat (80%) or convex (20%) and pigmentation was white. 50% of irregular-undulate colonies were with flat elevation and rest 50% with raised elevation. While all the punctiform-entire and filamentous colonies showed flat elevation. Like sample A, bacterial colonies of soil sample B were also white in colour and were positive in gram staining reaction.

In quantitative analysis, bacterial population was observed and was represented as colony forming unit (cfu/g of soil) and has been presented in Fig. 1. It was observed that bacterial population of soil sample B was always less than soil sample A. In first observation, mean bacterial population in sample A was $33.4\pm0.989\times10^{9}$ and in sample B it was $28.3\pm1.921\times10^{9}$. The mean bacterial population of sample A after 2nd, 3rd and 4th observation was $52\pm0.866\times10^{9}$, $22.5\pm1.50\times10^{9}$ and $42.1\pm1.724\times10^{9}$ and for sample B it was $49.4\pm1.40\times10^{9}$, $16.4\pm2.138\times10^{9}$ and $34.6\pm1.040\times10^{9}$. The bacterial population gradually declined from first to last observation and the change in population was found to be significant ($p < 0.001$).

A similar trend of decline in wet weight (mg/g soil) and biomass (mg/g soil) was observed between sample A and sample B (Figs. 2 and 3). Wet weight for sample B was $42.5\pm2.88\times10^{-3}$, $74.1\pm2.1\times10^{-3}$, $24.7\pm3.20\times10^{-3}$ and $52\pm1.56\times10^{-3}$ which was always significantly ($p < 0.001$) less than $50.1\pm3.04\times10^{-3}$, $78\pm2.25\times10^{-3}$, $33.75\pm2.25\times10^{-3}$ and $63.2\pm2.88\times10^{-3}$ for sample A. The biomass of sample A recorded was $10.02\pm0.61\times10^{-3}$, $15.6\pm0.45\times10^{-3}$, $6.75\pm0.45\times10^{-3}$ and $12.64\pm0.52\times10^{-3}$ and of sample B was $8.5\pm0.57\times10^{-3}$, $14.82\pm0.42\times10^{-3}$, $4.94\pm0.64\times10^{-3}$ and $10.4\pm0.31\times10^{-3}$. The decline in biomass of sample B over sample A was also found to be significant ($p < 0.001$).

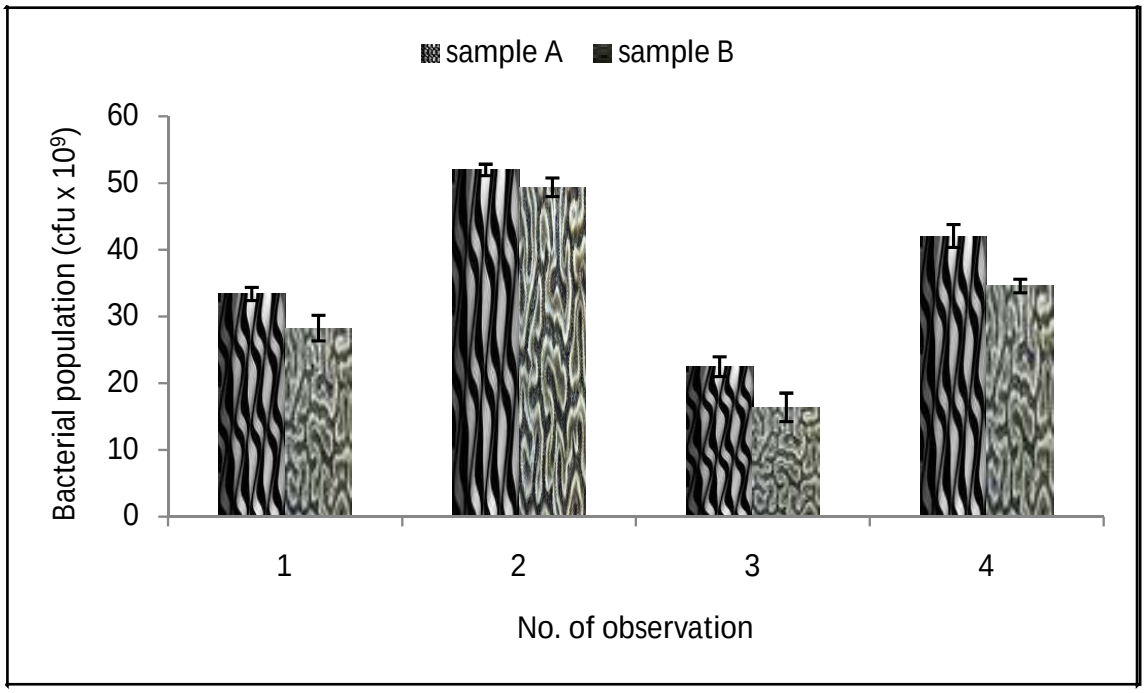

Figure 1 : Bacterial population (number per g soil) in sample A and sample B

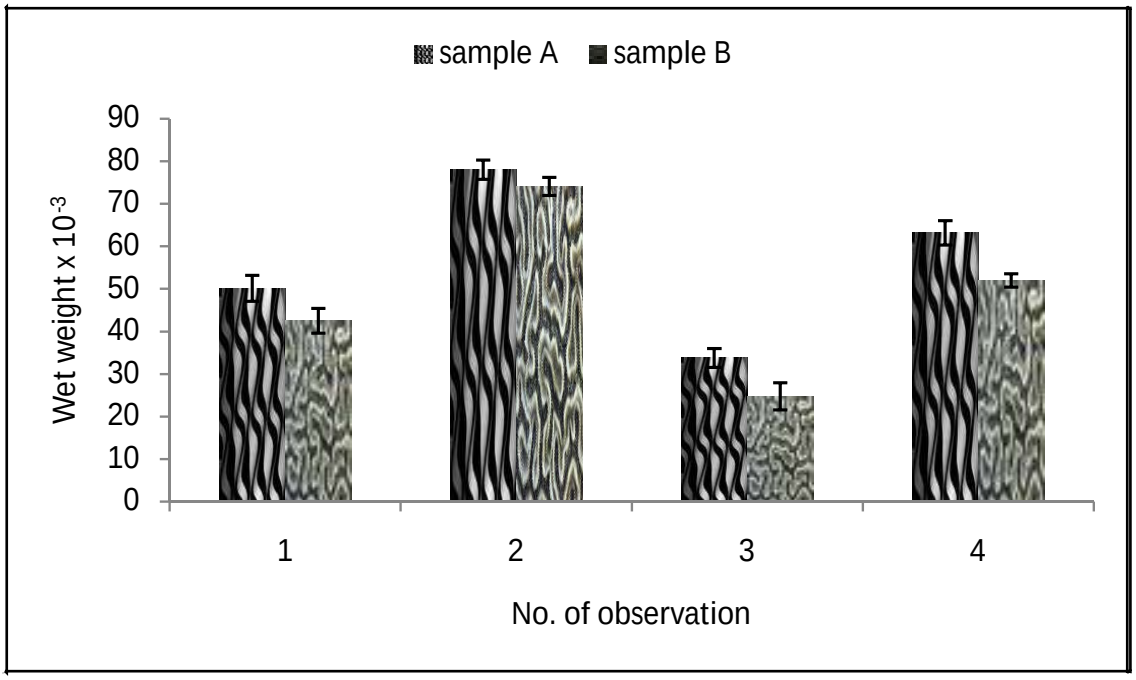

Figure 2 : Wet weight (mg/g soil) of bacterial population in normal soil and *Pathenium* affected soil

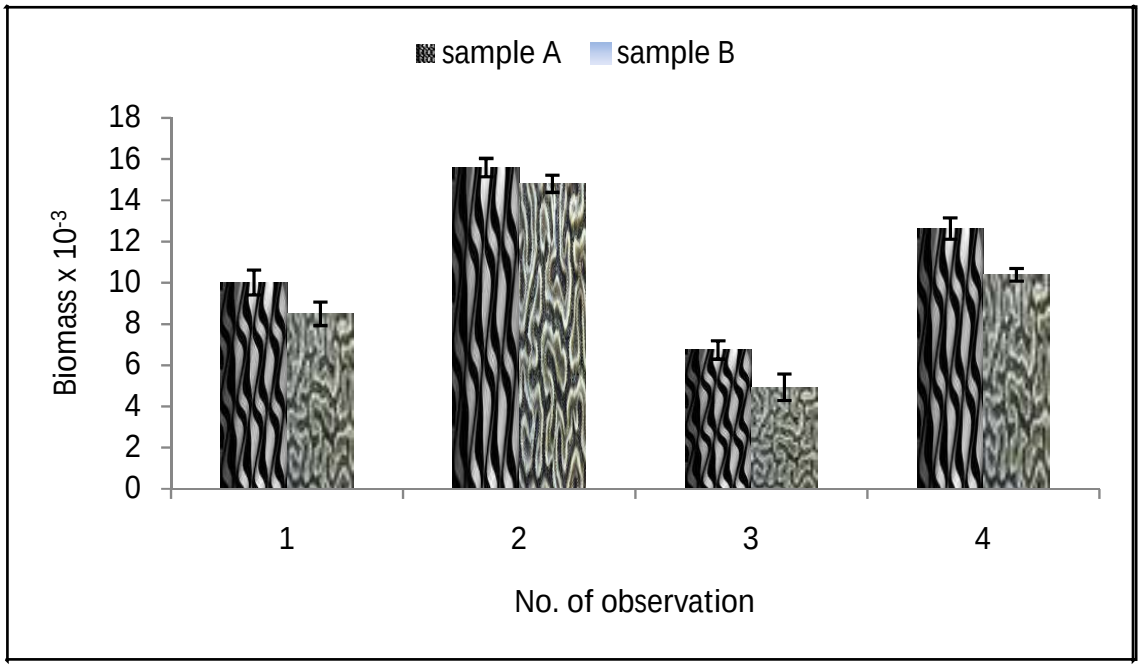

Figure 3 : Biomass (mg/g soil) of bacterial population in normal soil and *Parthenium* affected soil

Discussion

Parthenium hysterophorus L. is an aggressive and noxious weed, commonly known as congress weed has become a threat to the environment and biodiversity. The colony forming units of normal soil is always more than the *Parthenium* infested soil (Fig. 1). This could be attributed to the fertility of soil and fertile soil contains fantastic number of living microorganisms (Francis, 1951). The bacterial population of *Parthenium* infested soil showed decrease in colony forming units and this could be because of the difficulty in survival and multiplication of bacteria in soil containing decomposed material of weed or accumulation of toxic chemical, viz. parthenin, caffeic acid, vanillic acid, ansic acid etc. which are present in the weed. Chemical analysis has indicated that all plant parts including trichomes and pollen contains lethal toxin, parthenin from the chemical group of sesquiterpene lactones (Oudhia and Tripathi, 1998). Enumeration of bacterial population of soil samples showed variations in all observation and the variation could be due to seasonal changes. Large effect of seasonal changes in soil moisture, soil temperature and carbon input on soil microbial biomass and its activity has been reported by Ross (1987) which in turn, affect the ability of soil to supply nutrients to plants through soil organic matter turnover (Bonde and Roswall, 1987). Microbial biomass has been reported to vary seasonally in European soils (Patra *et al*., 1990). The resultant wet weight and biomass of bacterial colony revealed significant reduction (Figs. 2 and 3). Doran (1980) and Handrix *et al*. (1986) reported the microbial biomass and their activities in soil may fluctuate due to different soil management practices. Amendment of soil by FYM (field yard manure), sheep dung, green manure, rice straw and sewage sludge showed significant increase in available phosphorus content, microbial biomass and dehydrogenase activity in soil (Sindhu and Beri, 1986; Ghany *et al*., 1997; Tillak *et al*., 1986; Mukharjee *et al*., 1990; Harden *et al*., 1993 and Saha *et al*., 1995) while Miller (1973) and Kulkarni and Pushpa (1993) reported the adverse effect on soil microbial population and activities in sludge and weed amended soils respectively. Soil containing minerals and microorganisms are supportive for plant growth but if get contaminated becomes least supportive. Patrich *et al*. (1984) stated that decomposition of plant residue in soil may have either favourable or unfavourable effect on plant growth.

The results indicated that *Parthenium hysterophorus* imparts adverse effect on soil bacteria. The gram positive bacterial colony with punctiform-entire, shape and margin appeared to be resistant to *Parthenium* infestation in comparison to other bacteria. Hence, bacteria colony having such type of colony morphology can be used as bioindicator to indicate the level of pollution caused by weed *Parthenium* .

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