



Anthropogenic impacts on phytosociological features and soil microbial health of wetland plant, *Acorus calamus* L.: An endangered medicinal plant species of Kashmir valley

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ABSTRACT

Acorus calamus L., is currently severely endangered medicinal plant species in the Kashmir wetlands. It is crucial to comprehend the phytosociological and conservational state of the plant in its native environment due to the ensuing rise in humanistic impact on medicinal plant species in damaged and undisturbed sections of the Kashmir wetlands. The present study was conducted during the years 2019 to 2021; wherein impact of anthropogenic activities on phytosociological features and soil microbial health of *Acorus calamus*, was studied. In the present study it was observed the highest values of density ($4.166 \pm 0.21 \text{ m}^2$); frequency ($56.743 \pm 1.70\%$) and abundance ($7.374 \pm 0.02 \text{ m}^2$) were recorded at undisturbed site i.e Shallabugh wetland as compared to other wetlands. The Shallabugh Wetland had the highest overall bacterial count (34.00 ± 0.06) as well as *Vesicular Arbuscular Mycorrhiza* (VAM) spore population (g^{-1}) (10.56 ± 0.29), as per an analysis of the rhizospheric soil of *A. calamus*. The current findings may be valuable in creating conservation methods for the highly endangered medicinal plant species, *A. calamus*. In disturbed ecosystems where human endeavours like vacations, grazing, urbanisation, and transportation have an impact, plants have an effect on the phytosociological and soil microbiological patterns. The study thus highlights the necessity for optimum management practises for medicinal plants and wetlands that provide effective monitoring and regulation of human operations in wetland zones and prevent the incursion of present reserves.

Key words: Abundance; *Acorus calamus*; density; endangered; frequency; *Vesicular arbuscular mycorrhiza*; wetland plant

Acorus calamus L., often known as Sweet Flag, is a member of the Acoraceae family. The genus *Acorus* is named after Acoron (coreon = eye pupil), whereas the species *Calamus* is named after the Greek term Calamos (a reed). There are over 110 genera and over 1,800 species in the Acoraceae family. *Acorus calamus* is a species native to Central Asia, India and the Himalaya. (Dusek *et al.*, 2007). It thrives in temperate and sub-tropical climates. *A. calamus* possesses a large geographical distribution in India from Kashmir to the north east ascending to an altitudinal range of 1500-2200 m in the Himalayas. It is grown in Karnataka, Himachal Pradesh, Manipur, Uttarakhand, Jammu and Kashmir, Nagaland, Uttar Pradesh, Tamil Nadu and Andhra Pradesh and Maharashtra. It is locally known by the name Vai-gander. Its rhizome is cut into small circular pieces, dried and made into a garland and is as such sold in market. Traditionally the rhizome portion is used for treatment of cough, stomachache, swellings, fever, toothache, diarrhea, headache, loss of appetite and Joint pains, etc. (Bano *et al.*, 2017, 2022a).

The rhizome is also used as abortifacient, carminative, diaphoretic, febrifuge, stimulant and vermifuge (Devi *et al.*, 2014). It commercially occurs in both peeled and unpeeled forms. The term "vegetational

composition" refers to a metric for measuring species diversity and community distribution. As a result, identifying systematic kinds becomes easier. However, because the vegetational composition of an area is subject to change, it is a long-term procedure to provide conclusive data. These interactions produce the effects of biotic and abiotic stress on a community (Whittaker, 1970). Floristic composition and phyto-sociology have long been of principal interest especially of agrostologists and ecologists but work done on these parameters especially in wetland areas of Kashmir valley is sparse. In a study of *A. calamus*, anthropogenically-induced eutrophication is accompanied by changes in the structure and function of the entire wetland ecosystem (Vojtíšková, *et al.*, 2004). Although the optimal climatic parameters for *A. calamus* in terms of water and soil properties varied depending on growth stage like seed germination and seedling establishment. The overexploitation and overgrazing are the most significant variables in lowering plant species diversity (Jeon *et al.*, 2011). The habitat of the mentioned plant species had been severely damaged, particularly by filling of wetlands, which has resulted in its endangered status in India. The diversity and distribution of the herbaceous medicinal plant *A. calamus*, as well as the total bacterial count (CFU) of soil and (VAM) spore population (g^{-1}) of soil in the wetland areas of Kashmir, were the subjects of the current inquiry. Overgrazing, tourism, urbanisation, and traffic have been the major factors contributing to ecological degradation. Their effects on the soil environment and plant ecosystem have led to decreased vegetation cover, decreased levels of organic matter, and negative effects on the microbial health of the soil (Butzer, 2005; Bhat *et al.*, 2021). Anthropogenic activities have impact on physico-chemical properties of plant, water as well as on sediment (Bano *et al.*, 2021, 2022b, c). As no research on *A. calamus* ecological characteristics or rhizospheric soil health characteristics had been done. Therefore, this study was undertaken on such an economically important medicinal plant in the different wetlands of the Kashmir valley.

MATERIALS AND METHODS

Study of population dynamics: The present study was carried out during the year 2020-2021 in the Kashmir wetlands at four different sites viz., Hokersar wetland (Srinagar), Shalabugh wetland (Ganderbal), Manasbal lake (Ganderbal) and Anchar lake (Srinagar) on the medicinal plant *Acorus calamus*. Three sunsites were selected within each wetland (S1, S2 and S3, approximately 200meters apart from each other). The valley of Kashmir is situated in western Himalayan range, which extends between 32°-20' to 34°-54' N latitude and 73°-55' to 75°-35' E longitude, location of the study area is shown (Fig. 1). The identification characters used to confirm the identity of *A. calamus* include the plant's rhizomes and leaves.

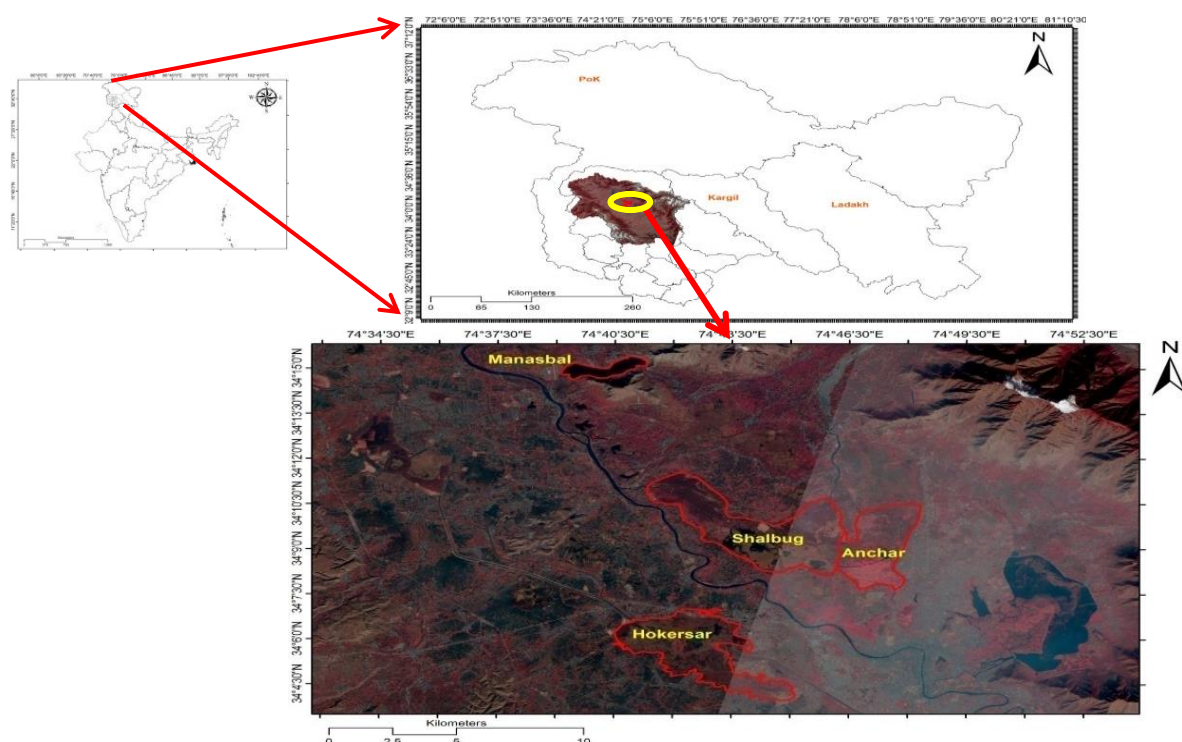


Fig. 1: Location map of the Manasbal, Shalabugh, Anchar and Hokersar wetlands of Kashmir valley

Quadrant analysis (field study): Quadrat analysis was conducted within the study sites. The analytical characters of the medicinal plant *A. calamus* were studied in terms of quantitative structures. These include frequency, density and abundance. The quadrat analysis was carried out in different regions of Kashmir wetlands viz. Hokersar wetland (Srinagar), Shallabugh wetland (Ganderbal), Manasbal Lake (Ganderbal) and Anchar lake (Srinagar) to study the population dynamics of the selected medicinal plant species. The total of six quadrants of size 10 m x10 m were laid down in each of the sub-locations. Within each of the 10m x 10m quadrant, five quadrants of size 1 m x 1 m were laid to note down the various ecological parameters.

Phytosociological parameters include:

	Number of quadrants in which the species occurred	
Frequency (%) =	$\frac{\text{Total number of quadrants studied}}{\text{Total number of quadrants studied}} \times 100$	
Density =	$\frac{\text{Total number of individuals of a species in all quadrants}}{\text{Total number of quadrants studied}}$	
Abundance =	$\frac{\text{Total number of individuals of the species in all the quadrants}}{\text{Number of quadrates in which species occurred}}$	

Estimation of total microbial count (bacterial) and Vesicular Arbuscular Mycorrhiza (VAM) Population from soil of Rhizosphere:

- The total microbial count was estimated using nutrient agar media utilising the serial dilution pour plate approach (Aneja, 1996). An electronic colony counter was used to count the colonies that grew on the media. The microbial count was expressed as colony forming unit per gram of soil (CFU/g soil). The serial dilutions of the soil samples were made up to 10^6 and 0.1 ml of diluted soil suspension was plated on Nutrient agar medium plates.
- The AM fungal spores were extracted from the soil using a wet sieve and decanting approach (Gerdeman and Nicolson, 1963), while the AMF spores were isolated using a sucrose gradient centrifugation technique (Daniel and Skipper, 1982) and then counted (Sylvia, 1994). For the identification of AM fungal spore, single spore or sporocarps were easily picked up from the sieves with the help of syringe needle and mounted on a glass slide with a drop of polyvinyl lacto phenol (PVL) and a cover slip was placed. The spores were identified by using dissecting microscope.

RESULTS AND DISCUSSION

Impact of anthropogenic activities on phytosociological parameters viz., density, frequency and abundance of *A. calamus*: The phytosociological variables are the most researched for features of any vegetation type. These features show that how plant communities change. The amount of damage that leisure and tourism do to plant growth in wetlands depends on a number of variables, including the type of infrastructure provided, the volume of usage of sites, the type of activity, visitor behaviour, and the time of year that it is utilised (Liddle, 1997 and Cole, 2004). As many species are already on the point of extinction, tourism has an especially detrimental effect on rare and fragile plants. The effects of tourism on rare flora, particularly that found in protected areas, remain unknown, despite the fact that there is evidence of these taxonomic regions' tourism's adverse environmental effects (Kelly *et al.*, 2003). In the present study the density, frequency and abundance at four wetlands of Kashmir valley the results revealed that among all the four sites viz, Hokersar wetland (Srinagar), Shallabugh wetland (Ganderbal), Manasbal lake (Ganderbal) and Anchar lake (Srinagar) the highest density of the plant *A. calamus* was shown at Shallabugh wetland ($4.17 \pm 0.21 \text{ m}^2$) and least average density was shown at Hokersar wetland ($1.42 \pm 0.02 \text{ m}^2$). The highest frequency and density was also shown at Shallabugh wetland ($56.74 \pm 1.70\%$) and ($7.37 \pm 0.02 \text{ m}^2$) and the least was recorded at Hokersar wetland ($31.50 \pm 0.23\%$) and ($5.583 \pm 0.06 \text{ m}^2$). (Table 1) (Fig.2). The lower values of abundance, density and frequency at Manasbal Lake and Anchar Lake, particularly at Hokersar wetland may be because of high biotic interference

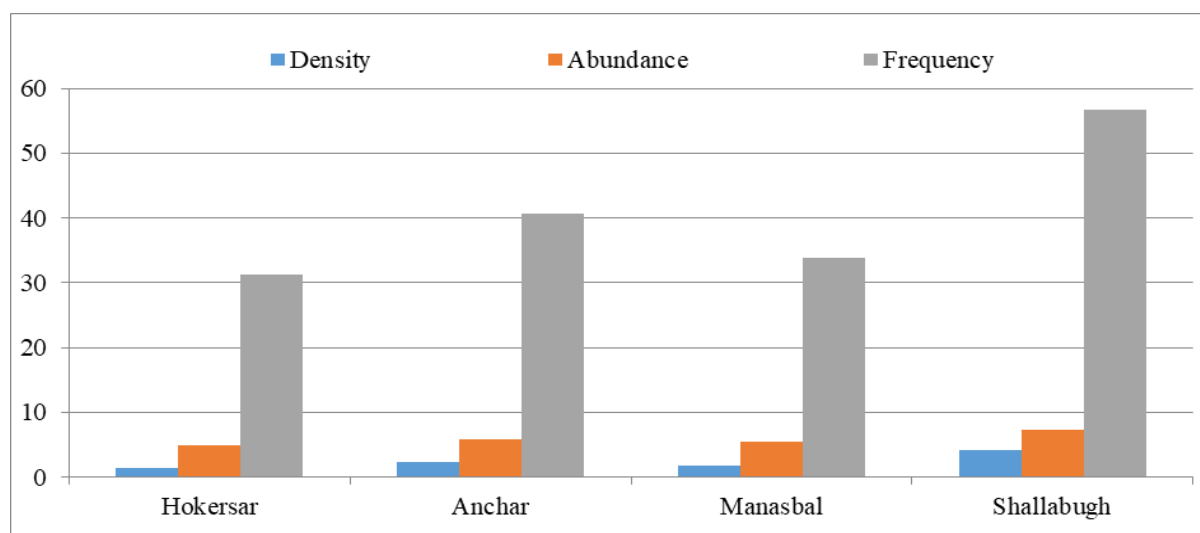


Fig. 2. Frequency, density and abundance of *A. calamusat* at four locations of Kashmir.

like grazing. Grazing is one of the most important disturbances in terms of both production and vegetation dynamics (Baba *et al.*, 2017). Many biotic pressures on grasslands and other forest vegetation types, including grassland total biomass, have been the subject of extensive investigations (Baba *et al.*, 2017). Our knowledge of how grazing, deforestation, tourism, and other factors affect vegetation has greatly benefited from these studies. In addition, human impact, economic exploitation, agricultural needs and, forest fires are all major sources of disturbance (Singh and Singh, 1992). At the Shallabugh wetland in the Kashmir valley, the average frequency, density, and abundance of *A. calamus* was higher (Table 1). Therefore, our study shows that disturbed areas have increased anthropogenic interference and biotic stress at the herbaceous level. Similar observations were made by Kukshal *et al.*, (2006); Mushtaq and Pandit (2010); Baba *et al.*, (2017). There are evident direct effects of recreation and tourism such as clearing vegetation for infrastructure, transportation systems and sometimes severe horseback riding on plant biodiversity and vegetation communities, including many rare and threatened plants (Pickering *et al.*, 2017).

Table 1: Impact of biotic stress on average density, frequency% and abundance of *A. calamus* L, at four locations of Kashmir wetland

Sites	Sub-site	Density (m ²)	Frequency (%)	Abundance (m ²)
Hokersar (Srinagar)	S1	1.567±0.09	31.517±0.24	5.120±0.01
	S2	1.253±0.02	30.567±0.20	4.250±0.01
	S3	1.420±0.02	31.500±0.23	5.583±0.06
Mean±SE		1.413±0.04	31.195±0.22	4.984±0.01
Anchar Lake (Srinagar)	S1	2.327±0.01	40.567±0.15	5.767±0.03
	S2	2.367±0.03	40.333±0.29	5.867±0.02
	S3	2.520±0.01	41.300±0.12	6.107±0.01
Mean±SE		2.405±0.02	40.733±0.18	5.914±0.02
Manasbal (Ganderbal)	S1	1.813±0.01	33.647±0.17	5.407±0.01
	S2	1.687±0.01	32.820±0.04	5.140±0.01
	S3	2.123±0.02	35.250±0.03	5.977±0.01
Mean±SE		1.874±0.03	33.906±0.12	5.508±0.01
Shallabugh (Ganderbal)	S1	4.067±0.07	57.190±0.54	7.163±0.04
	S2	4.103±0.15	56.737±1.03	7.327±0.01
	S3	4.327±0.32	56.303±2.52	7.633±0.31
Mean±SE		4.166±0.21	56.743±1.70	7.374±0.02
C.D(p<0.05)		0.390	0.450	0.320

Impact on total bacterial count *Vesicular arbuscular mycorrhiza* (VAM) population of rhizospheric Soil of *A. calamus*:

The most significant purpose of soil microorganisms in ecosystems is to promote organic matter decomposition, synthesis, and release inorganic forms that plants can utilise (Setiadi, 1989). The soil is home to the majority of bacteria in the terrestrial ecosystem. Bacteria are by far the most common type of soil microorganism. There are 10^6 – 10^8 bacteria cells per gram of soil in productive soil. Some types of soil bacteria (nitrogen-fixing and phosphate-solubilizing bacteria) can be used as biofertilizers. Symbiotic or mutually beneficial connections have developed between some plant and microbial species. In the root of legumes, *Rhizobium*, is home to nitrogen-fixing bacteria that can infect the root and extract sugars from the plant. According to Zahran *et al.*, (1999), in exchange, they convert vast amounts of dinitrogen (N_2) from the environment into forms that the plants can consume. Plants can serve as factories for production of medicinal compounds with immense trade value. Tripathi, and Srivastava (2020), studied *Vesicular arbuscular mycorrhizal* fungal diversity in sixteen medicinally important plants belonging to different families and reported presence of 12 VAM fungi associated with them. In wetland ecosystems, AMF can improve the tolerance of wetland plants to heavy metals by changing the structure and composition of rhizosphere bacterial communities (Wei, *et al.*, 2023). In the present study highest bacterial count CFU (g^{-1}) in rhizospheric soil of *A. calamus* in *Hokersar* wetland and Manasbal Lake was obtained at site S3 and least was obtained at site S1. However, in Anchar Lake highest bacterial count CFU (g^{-1}) in rhizospheric soil of *A. calamus* was obtained at site S3 and least was at site S2, while as in Shallabugh, highest bacterial count CFU (g^{-1}) was obtained at site S1 and least was at site S3. Among the sub sites, sub site S3 seem to be the slightly better one in three wetlands as compared to Shallabugh wetland (Table 2 and Fig. 3). It may be because of good amount of organic matter at site S3. As the most prevalent type of soil microorganism is by far bacteria. In productive soil, there are 106–108 bacterium cells per gram of soil. Bhardwaj *et al.*, (2023) also identified several bacterial species in the rhisosphere of *A. calamus* and reported that these bacterial species are good for plant growth permoting traits viz. phosphate solubilization, siderophores, and ammonia production, protease, chitinase and cellulase activity as well. Nitrogen-fixing and phosphate-solubilizing bacteria are two examples of soil bacteria that can be employed as biofertilizers. Some plant and microbial species have evolved symbiotic or mutually advantageous relationships. Nitrogen-fixing bacteria can infect the root of legumes, *Rhizobium*, and cause the plant to lose carbohydrates as a result of the infection. In exchange, according to Zahran *et al.* (1999), they transform enormous amounts of dinitrogen (N_2) from the environment into forms that the plants can absorb.

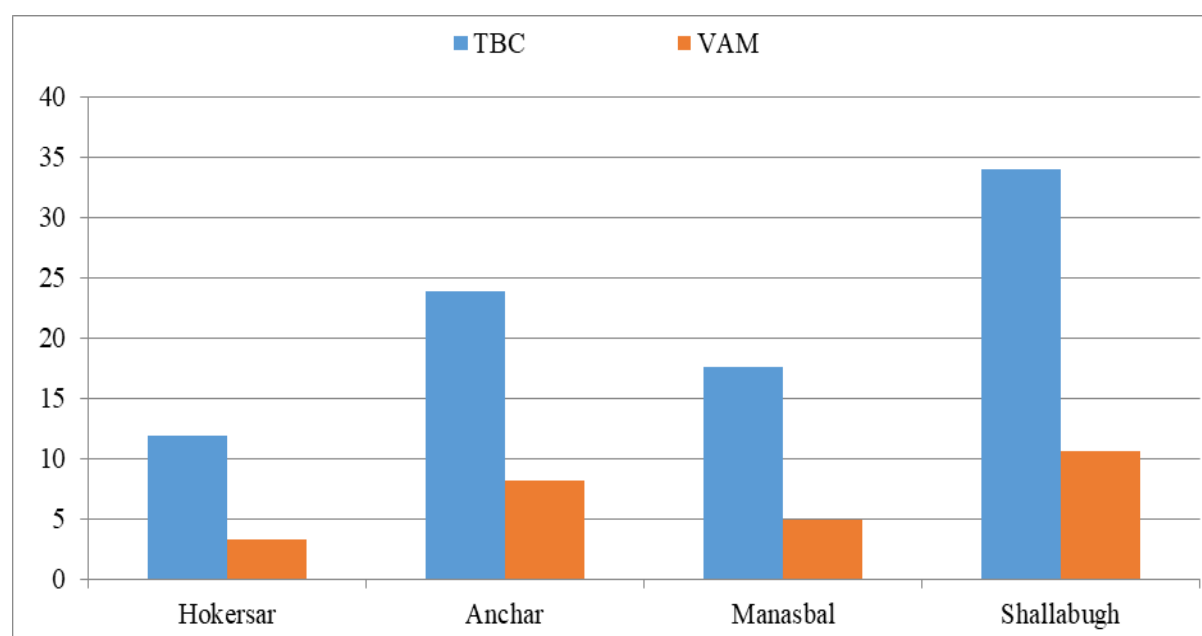


Fig 3. Total Bacteria Count CFU (g^{-1}) and VAM spore population (g^{-1}) at four sites

The highest total average bacterial count CFU (g^{-1}) in rhizospheric soil of *A. calamus* among four wetland of Kashmir valley viz. *Hokersar* wetland (Srinagar), *Shallabugh* wetland (Ganderbal), *Manasbal* lake (Ganderbal) and *Anchar* lake (Srinagar) was found at the undisturbed site i.e. *Shallabugh* wetland (34.00 ± 0.06) and the lowest was observed at *Hokersar* wetland (11.89 ± 0.71) (Table 2). This is because of the higher amount of organic carbon present at the undisturbed site. Similar results were reported by Ogunmwoyoni *et al.*, (2008) while studying the soil samples from sources other than wetlands. They reports reported that total bacterial count of

Table 2 Impact of biotic stress on bacterial count of soil at four locations of Kashmir wetlands

Site	Sub sites			Mean±SE
	S1	S2	S3	
Hokersar	10.68±0.21	11.68±0.31	13.33±0.42	11.89±0.71
Anchar Lake	24.33±0.01	21.33±0.02	26.00±0.01	23.89±0.01
Manasbal	16.00±0.23	17.01±0.34	19.68±0.51	17.56±0.36
Shallabugh	35.00±0.09	35.33±0.04	31.68±0.12	34.00±0.06

each soil sample ranged from 9.5×10^7 colony forming units (cfu) per gram of soil, 8.0×10^5 cfu/g of soil, 6.5×10^5 cfu/g of soil respectively. The highest *Vesicular Arbuscular Mycorrhiza* (VAM) spore population (g^{-1}) in soil was observed at Shallabugh wetland (10.56 ± 0.29) and the lowest *Vesicular Arbuscular Mycorrhiza* (VAM) spore population (g^{-1}) of soil were observed at Hokersar wetland (3.22 ± 0.49). (Table 2) (Fig.3). At all disturbed areas, the total VAM population was found to be lower as compared to undisturbed area i.e Shallabugh wetland. Numerous physical and biological elements influence the VAM spore population. Similar observation was made by Sieverding *et al.*, (1991) and Jamiołkowska *et al.*, (2018). Shallabugh had the most bacteria per gram of soil, whereas Hokersar, Manasbal, and Anchar had the fewest bacteria per gram of soil, correspondingly. At disturbed areas, grazing, tourism, etc. are more prevalent, which may result in reduced organic matter and aeration in the soil, both of which have a positive correlation with the growth of the microorganism population. Similar work made by other workers (Bhattarai *et al.*, 2015). The highest mean *Vesicular Arbuscular Mycorrhiza* (VAM) spore population (g^{-1}) of soil was (10.56 ± 0.29) at Shallabugh (Table 3). Similar work was reported by other authors (Sarkar *et al.*, 2014). Bacterial and VAM spore population at Shallabugh wet land were statistically significant. These results are similar with findings of Torsvik *et al.*, (2002). The edaphic and climatic conditions in which every species competes for survival are related to the distribution of *Vesicular Arbuscular Mycorrhiza* (VAM), and the best-suited species quickly multiplies and establishes itself in soil, especially when soil is under anthropogenic pressure. According to Dickman *et al.* (1984), Porter *et al.* (1987), and Lakshmipathy *et al.* (2003), soil acidity is a key factor influencing spore germination and may also have an impact on the dispersion of AM fungi.

Table 3 Impact of biotic stress on VAM spore population (g^{-1}) of soil at four locations of Kashmir wetlands

Site	Sub sites			Mean±SE
	S1	S2	S3	
Hokersar	3.01±0.57	3.67±0.54	3.00±0.55	3.22±0.49
Anchar Lake	7.00±0.32	9.00±0.42	8.33±0.15	8.11±0.18
Manasbal	5.00±0.59	4.00±0.57	5.67±0.52	4.89±0.58
Shallabugh	10.33±0.32	9.33±0.34	12.00±0.43	10.56±0.29

CONCLUSION

The findings of this study demonstrate that biotic stress causes decreased vegetation, which affects soil health and also the phytosociological characteristics of the commercially important medicinal plant species, *A. calamus* of the Kashmir. This study urges the use of the best wetland management strategies to prevent the encroachment of current reserves while effectively monitoring and regulating human activities in and around wetland ecosystems. In addition to preserving wetland productivity and long-term soil fertility, restoration programmes would offer a way to prevent the irreversible degradation of medicinal plant species and wetland soil systems. They would also offer a way to safeguard the livelihoods of people who depend on wetland resources.

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