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Vegetation development and diversity of Arbuscular Mycorrhizal fungi associated with some plants of abandoned cropland in J. P. University Campus, Chapra Bihar

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ABSTRACT

The floristic composition and live shoot biomass estimation of a grassland community developed in abandoned cropland after about nine years at Jai Parkash University Chapra (25°36' - 26°15' N lat. and 84°24' - 85°15' E long.) were studied. The floristtic compositoin of the grassland community was composed of 14 species. *Dicanthium* annulatum and *Cynodon dactylon* were the dominant species. The IVI values were 43.66 and 70.99 for *Cynodon dactylon* and *Dicanthium annulatum,* respectively. The maximum spore population was observed in the species, *Clerodendrum infortunatum Linn.* (155/ 100 gm of soil) and minimum in *Citrus lemon (L.*) (10/ 100 gm of soil). maximum relative abundance (RA) was recorded in *Glomus intraradices* (33.3 %) and minimum RA was recorded in *Acaulospora delicata, Acaulospora morrowiae* etc (0.16%).

Keywords - Species composition, Importance value index, Arbuscular mycorrhiza, Root colonization, Relative abundance

INTRODUCTION

Ecologically a grassland may be defined to be the land on which graminoid (poaceae) species are dominant. Grassland as a whole encompasses the potential natural vegetation of 25% of the earth's land surface (Shantz, 1954), and accounts for about 16% (1.89 ×1010 t/ ha^{-1}) of the net primary productivity of plant community (Whittaker and Likens, 1973). According to Singh (1987), grasslands in India occupy 39.81 per cent (12121 thousand ha) area of the total Indian sub-continent. Whyte (1968) reported that grasslands occur on almost all soil types and their distribution is predominently governed by climatic factors. The tropical grasslands in India are seral in nature due to reccuring biotic operation such as grazing, fire and scraping (Neeraj *et al*., 2004). Since 2006 cultivation of crops has been banned in the J. P. University Chapra, campus and the campus is now a fallow land. Only within nine years the vegetation has developed which is a grassland type. According to Bignal (1996) and Kleijn *et al*. (2006) some

agricultural and agroforestry systems that shape cultural landscape have been recogonized which need conservation relevance including biodiversity, habitat and aesthetic values. The variable ecosystems would be lost if agricultural use is abandoned. There are negative as well as positive effects of abandonment of agricultural land. Reduction of heterogeneity and promotion of vegetation homogenisation associate, increase fire frequency, soil erosion and desertification forms the negative effects of abandonment (Jose *et al*. 2007) whereas, revegetation, forest plantation, water retension, soil recovery and nutrient cycling are its positive effects. A symbiotic association of a fungus and roots of higher plants was discovered by Franciszek Kamienski, and later on a Polish mycologist Frank (1985) coined the term "Mycorrhiza" to this association. Mycorrhiza belonging to most commonly occurring soil microorganisms of the world is considered as a fundamental part of the plant, as 95 % of all plant species could not servive in nature without it. AM fungi consist of intra and extraradical structures. The intraradical structures are arbuscules, vesicles and intraradical hyphae. The extraradical structures are extraradical hyphae, spores and auxillary cells. Arbuscular mycorrhizae (AM) are regarded as a mutualistic association in which plant provides the fungus with assimilates in exchange for mineral nutrients and water (Smith and Read 1997). In natural communities, approximately 80% of higher plants are obligatorily dependent on fungal associates and 18% typically non mycorrhizal (Trappe, 1987).

Therefore, the aim of the present study was to examine the species composition, live shoot biomass, abundance of arbuscular mycorrhizal fungi, extent of root colonization and isolation frequency of AMF of grassland vegetation developed after nine years of abandonment of cultivation in the J. P. University Campus, Chapra, Bihar.

MATERIALS AND METHODS

Description of Study sites

The present study was conducted during 2015 – 2017 in rainy season in the nine-year-old abandoned cropland of J. P. University, Chapra Campus which is spread in about 240 ha land. The study area is situated between $25^{0}36'$ - $26^{0}15'$ N lat. and $84^{0}24'$ - $85^{0}15'$ E long. The maximum temperature values ranged from 15.4^{0} to 44.5^{0C}.

Field Method and Vegetation Analysis:

Harvest method of Odum (1960) was employed for the estimation of phytosociological characteristics and plant biomass. Ten quadrats of 50 cm X 50 cm sizes were randomly harvested 1cm above the ground surface during the last week of months (2015- 2017) in rainy season. The samples were packed in polyethylene bags separately and brought to the laboratory for identification (Muller Dombois and Ellenberg 1974). Identification of all the species were made in consultation with various regional and national flora books i,e The Botany of Bihar and Orrisa (Haines, 1921-25) ; Supplement to the Botany of Bihar and Orrisa (Mooney, 1950). The phytosociological observations were made. The species present in quadrats were noted and their numbers were counted individually/tiller of each species. The vegetation data were quantatively analysed for frequency, density, abundance and dominance following Curtis and McIntosh (1950). Relative ferquency, relative density and relative biomass were determined following Phillps (1959), for dominance shoot biomass data were used. The Importance Value Index (IVI) was the sum of relative frequency, relative density and relative dominance. Following formulae were used Misra (1968).

$$\text{Relative frequency} = \frac{\text{Number of occurrences of the species}}{\text{Number of occurrences of all species}} \text{X } 100$$

$$\text{Relative density} = \frac{\text{Number of occurrences of a species}}{\text{Number of occurrences of all species}} \text{X } 100$$

$$\text{Relative biomass} = \frac{\text{Shoot biomass of a species}}{\text{Total shoot biomass of all species}} \text{X } 100$$

Important value index = Relative frequency+ Relative density+ Relative dominance

Root sampling:

In the months of rainy season of 2015 – 2017 fine roots of plants growing in the campus of J. P. University were collected from different plants such as *Calotropis gigantea (L.) Ait.*, *Ziziphus mauritiana Lamm.*, *Croton sparciflorus (L.), Parthenium hysterophorus L.*, *Dalbergia sissoo Rox. Ex D*c. etc. Roots were collected randomly from a depth of 0- 30 cm. After bringing these plant samples to laboratory the roots were separated and further processing was done.

Estimation of root colonization:
Roots were washed throughly to remove attached soil particles. The cleaned roots were cut into 1 cm long piece and were fixed in formalin acetic acid (FAA) according to the procedure described by Phillips and Hayman (1970). The roots were boiled in 10% KOH for 1 hr, acidified with 5N HCI and stained for 24 hr with 0.5 % tryphan blue. Each root was divided into 12 1cm long segments, which were then cleaned, stained and were arranged on slides. The slides were observed under compound microscope to score for any structures associated with mycorrhizal fungi like hyphae, vesicle, arbuscules, or hyphal coil in each segment. The percentage of AM fungal colonization was assessed by using the formula :

$$\text{Percentage of colonization} = \frac{\text{Number of root segments infected}}{\text{Total number of root segments observed}} \times 100$$

Spore extraction :
Separation of AM fungal spores from rhizospheric soil of each plant was done by using wet sieving and decanting method proposed by Gerdmann and Nicolson (1963) from the 100 gm of soil sample. Soil samples were collected randomly using three replicates. All the samples were sieved (< 2mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample. The root soil mixture was vigorously mixed with a glass rod for 30 seconds. The suspension was passed through 250µm, 150µm, 98µm and 75µm sieves. The material remaining on the sieve was washed into beakers. After settlement of the heavier particles, the supernants was filtered through gridded filter papers. Each filter paper was spread on to a glass plate and scanned under stereo microscope (Olympus SZ2-ILST). Intact and crushed spores were counted. AM fungal spores from the filter paper were picked up using a wet needle and mounted in Polyvinyl alcohal lactophenol (PVLG) on a glass slide and identified under a compound microscope (Olympus BX41) and photographed (Nikon eclipse 200). ldentification was based on spore morphology and sub cellular characters (Schenck and Perez, 1990).

Relative abundance and Isolation frequency of occurrence of AM fungi :
Relative abundance of occurrence of AM fungi was calculated dividing the number of soil samples that possess spores of particular species with the total number of soil samples screened and multiplied by 100. Isolation frequency was calculated dividing the number of soil samples possessing spores of a particular species with the total number of soil samples analyzed and multiplied by 100.

$$RA = \frac{\text{Number of spores of a species / genus}}{\text{Number of spores of all species / genus}} \times 100$$

$$IF = \frac{\text{Number of soil samples possessing spores of a particular species}}{\text{Total number of soil samples analyzed}} \times 100$$

RESULTS AND DISCUSSION

The present study deals with the phytosociological attributes of an openly grazed vegetation developed after nine years of abandonment of cultivation in the University campus of Jai Prakash University, Chapra. The maximum number of species were recorded 14 at study sites. In 2015 relative frequency (RA) was maximal for *Cynodon dactylon, Desmodium triflorum* and *Elusine indica* (10.94) and minimal for *Evolvulus nummularius* (3.48). Relative density (RD) was maximal for *Cynodon dactylon* (19.23) and minimal for *Evolvulus alsenoid* (1.43). Maximum relative biomass (RB) was recorded for *Dicanthium annulatum* (21.77) and minimal for *Evolvulus alsenoid* (1.31). Maximum importance value index (IVI) was recorded for *Dicanthium annulatum* (48.46) and minimum for *Evolvulus alsenoid* (6.90) (Fig.1). In 2016 maximum relative frequency was recorded for *Cynodon dactylon* and *Desmodium triflorum* (12.95) and minimal for *Euphorbia hitra* (3.87). Maximum relative density was recorded for *Dicanthium annulatum* (24.37) and minimal for *Croton sparciflorus* (0.53). Maximum relative biomass was recorded for *Dicanthium annulatum* (30.90) and minimum for *Euphorbia hitra* (1.36). Maximum IVI was recorded *Dicanthium annulatum* (67.73) and minimal for *Euphorbia hitra* (5.78) (Fig.2). In 2017 maximum relative frequency was recorded for *Dicanthium annulatum* (13.67) and minimal for *Evolvulus nummularia* (0.811). Relative density was maximal for *Cynodon dactylon* (23.93) and minimal for *Evolvulus nummularia* (0.05). Maximum relative biomass was recorded for *Dicanthium annulatum* (25.87) and minimal for *Evolvulus nummularia* (0.32). Maximum IVI was recoded for *Dicanthium annulatum* (70.99) and minimal for *Evolvulus nummularia* (0.906) (Fig. 3). The community

formed in 2015 was *Cynodon dactylon - Dicanthium annulatum* ; in 2016 was *Dicanthium annulatum - Cynodon dactylon* and in 2017 was *Dicathium annulatum - Cynodon dactylon*.

Colonization was characterized by the presence of hyphae, arbuscules, vesicles and hyphal coil. Percentage colonization was maximal for *Parthenium hysterophorus L.* and *Lantana camara Var.* (100%) and minimal for *Ricinus communis L*. (44.4%). Both Arum and Paris type morphologies were observed (Table 1). Twenty five AM fungal species of five genera viz, *Glomus , Acaulospora, Scutellospora, Sclerocystis* and *Entrophospora* were recovered from rhizosphere soils of the study sites. *Glomus* (14 species) was the dominated genus followed by *Acaulospora* (8 species), *Scutellospora* (1 species), *Sclerocystis* (1 species) and *Entrophospora* (1 species). The highest spore density was recorded in the plant species of *Clerodendrum infortunatum Linn*. (155/ 100 gm of soil) belongs to the family *Lameaceae.* The lowest spore density was recorded in *Citrus lemon (L.)* (10/ 100 gm of soil) belongs to the family *Rutaceae*. Twelve plant species of J.P. University of Chapra, campus were analyzed for mycorrhizal density, diversity, relative frequency (RA%), isolation frequency (IF%) etc in the present study. The maximum relative abundance (RA) was recorded in *Glomus intraradices* (33.38%) and minimum RA was recorded in *Acaulospora delicata, Acaulospora morrowiae, Acaulospora undulata, Glomus aureum, Glomus multicaule and Scutellospora reticulata* (0.16%). Highest isolation frequency (IF) was recorded for *Glomus fasiculatum* (100%) and lowest IF was recorded for *A. delicata, A. denticulata, A. morrowiae, A. scrobiculata, A. undulata, G. aureum, G. multicaule. G. pustulatum, Scutellospora reticulata* and *Sclerocystis rubiformis* (8.33%) (Table 2). Higher numbers of *Glomus* species were recorded in the present study. These observations are in consistence with Mosses (1990).

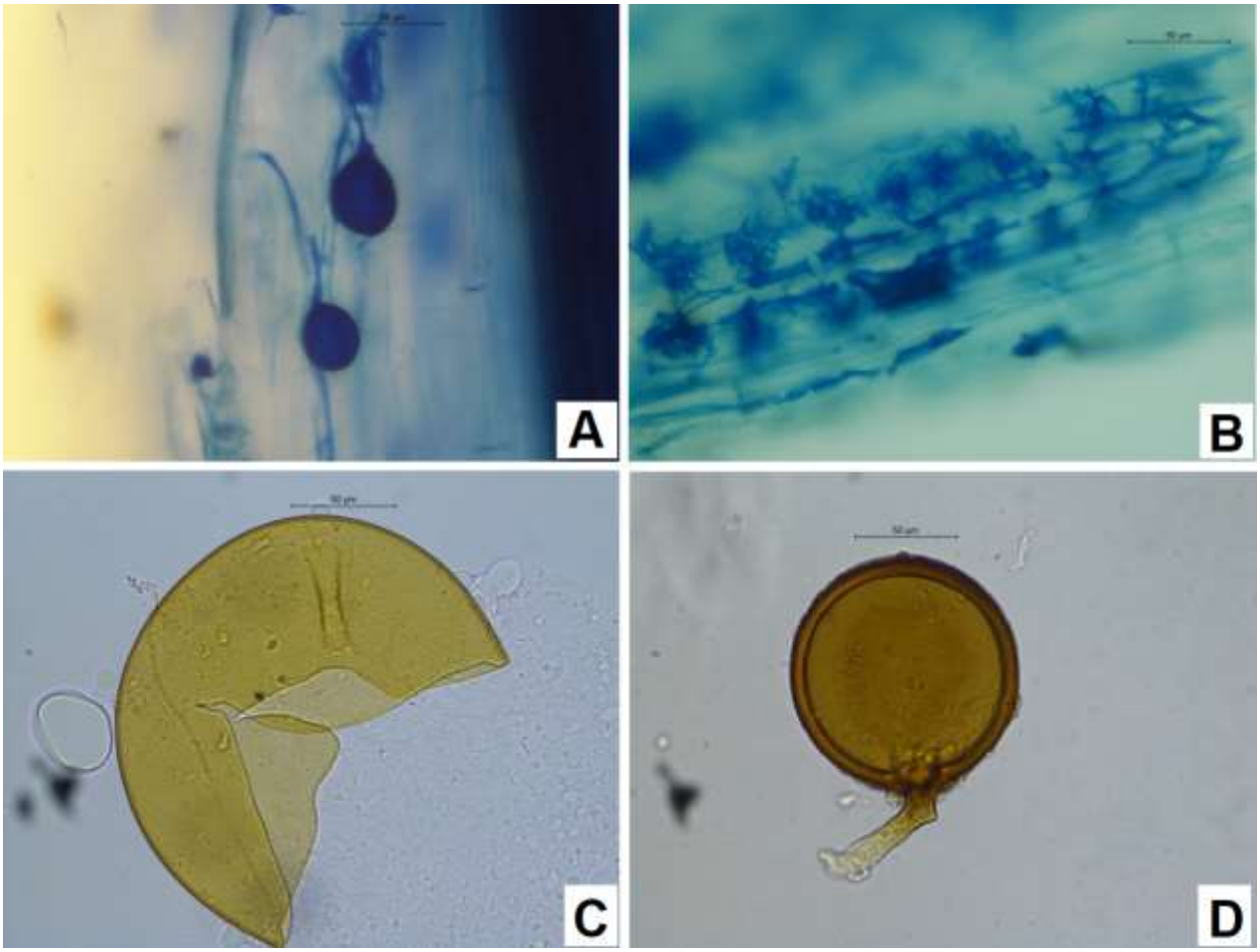

Fig. 4: A= Vesicles of *Parthenium hysterophorus L.*, *B*= Arbuscules of *Parthenium hysterophorus L.*
C = *Aculospora laevis*, D = *Glomus diamorphicum*

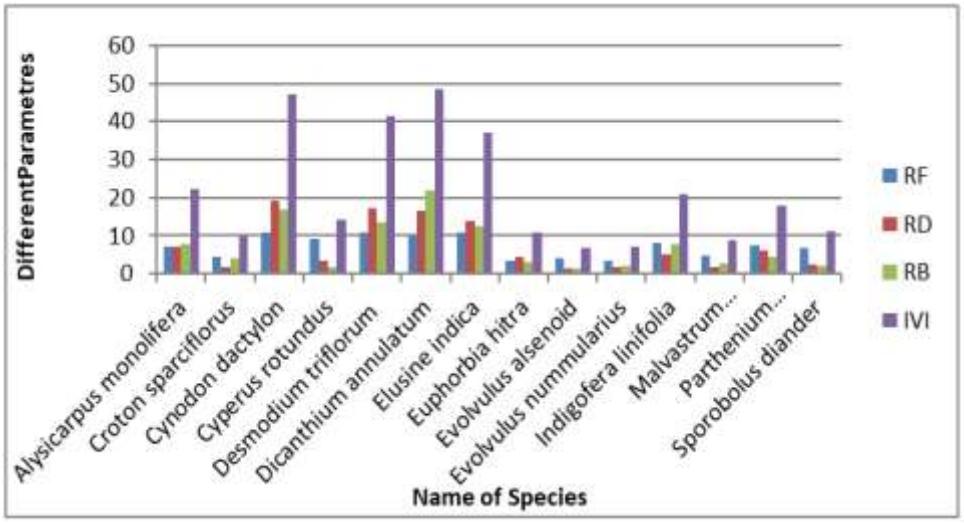

Fig. 1: Comparision between species and different parameters (2015)

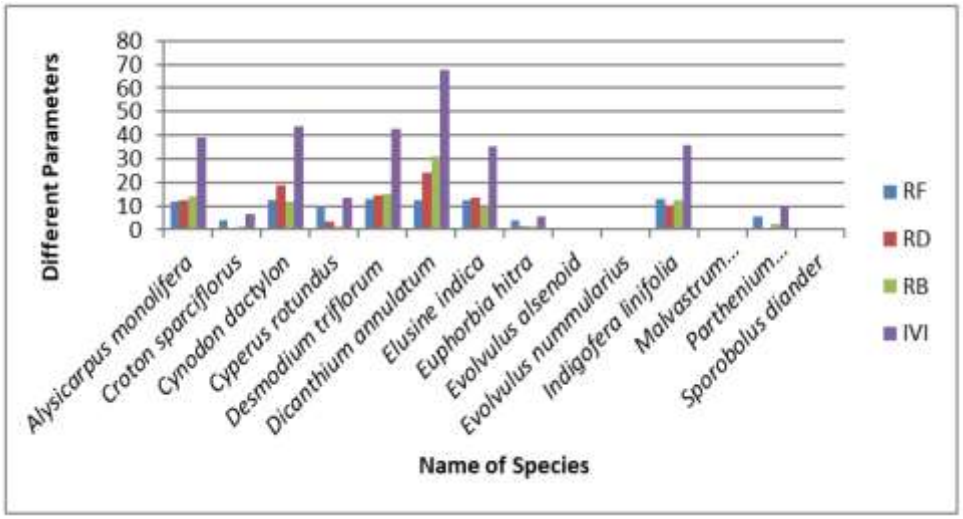

Fig . 2 Comparision between species and different parameters (2016)

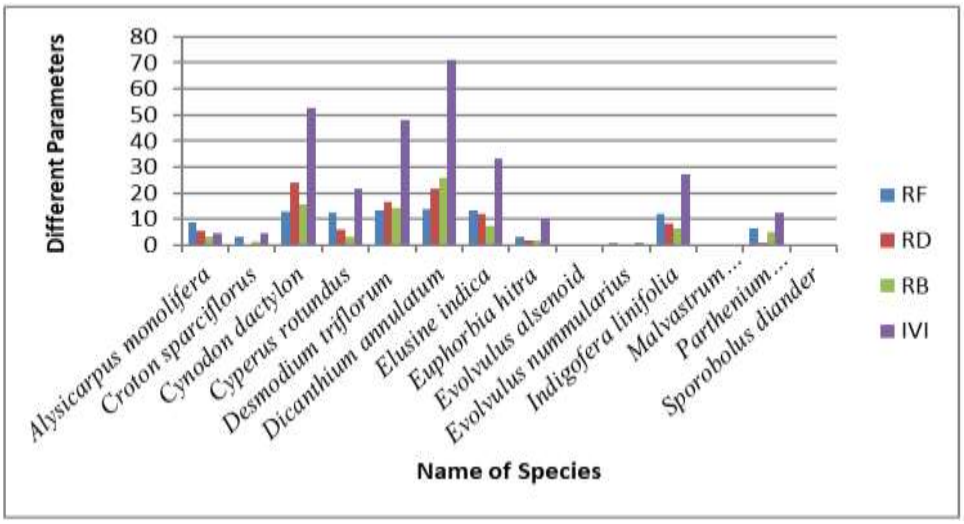

Fig. 3 Comparision between species and different parameter

Table l: AM association with roots and AM spores present in rhizospheric soil

Sr no	Plant name	Colonization (%)	Spore density per 100 gm soil
1	*Calotropis gigantea (L.) Ait.*	97.7 ± 4.8 ±2.8	78.33
2	*Citrus lemon (L.)*	72.2 ± 4.8 ± 2.8	10
3	*Croton sparciflorus (L.)*	94.4 ± 9.64 ± 5.5	113.33
4	*Azadirachta indica A. Juss.*	63.8 ± 4.7 ± 2.7	88.33
5	*Clerodendrum infortunatum Linn.*	77.7 ± 20.9 ± 12.10	155
6	*Ziziphus mauritiana Lamm.*	72.2 ± 4.8 ±2.8	93.33
7	*Parthenium hysterophorus L.*	100 ± 0 ± 0	125
8	*Dalbergia sissoo Rox. Ex Dc.*	63.8 ± 4.7 ± 2.7	73.33
9	*Phoenix dactylifera L.*	47.16 ± 17.3 ± 10	38.33
10	*Lantana camara Var.*	100 ± 0 ± 0	48.33
11	*Ricinus communis L.*	44.4 ±4.84 ±2.8	86.66
12	*Psidium guajava L.*	52.7 ±4.7 ± 2.7	128.33

Table 2: Relative abundance (RA%) and Isolation frequency (IF %) of AM fungi.

Sr no	AM Fungal Species	RA (%)	IF(%)
1	*Acaulospora delicata*	0.16	8.33
2	*Acaulospora denticulata*	0.16	8.33
3	*Acaulospora laevis*	1.30	25
4	*Acaulospora morrowiae*	0.16	8.33
5	*Acaulospora scrobiculata*	0.32	8.33
6	*Acaulospora spinosa*	0.81	16.66
7	*Acaulospora undulata*	0.16	8.33
8	*Acaulospora (unidentified)*	0.49	16.66
9	*Entrophospora*	1.14	16.66
10	*Glomus aggregatum*	2.94	33.33
11	*Glomus aureum*	0.16	8.33
12	*Glomus claroideum*	13.74	91.66
13	*Glomus clarum*	0.32	16.66
14	*Glomus dimorphicum*	0.98	25
15	*Glomus etunicatum*	6.21	33.33
16	*Glomus fasiculatum*	26.84	100
17	*Glomus geosporum*	1.14	25
18	*Glomus hoi*	0.65	25
19	*Glomus intraradices*	33.38	91.66
20	*Glomus macrocarpum*	1.80	33.33
21	*Glomus mosseae*	5.72	50
22	*Glomus multicaule*	0.16	8.33
23	*Glomus pustulatum*	0.81	8.33
24	*Scutellospora reticulata*	0.16	8.33
25	*Sclerocystis rubiformis*	0.16	8.33

The present investigation deals with the floristic composition of the vegetation in rainy season of Jai Prakash University campus, Chapra. Similar findings were also reported in grasslands of India Singh and Ambasht, (1975), Abdar (2013) and Bark and Misra (1997). However in Indian grasslands such as at Kurushetra density values ranged from 471to 2143 m-2 (Singh and Yadava, 1974), in Varanasi values ranged from 1963 to 11055 m-2 (Singh, 1967), in Ujjain values ranged from 104 to 862 m^{-2} (Misra, 1973) and in Ratlam values ranged from 21to 4700 m-2 (Billore, 1973). Pandeya (1974) has reported live shoot biomass value for *Cenchrus ciliaris* dominated grassland from 0 to 228 gm-2, at Rajkot, India. Kumar and Joshi (1972) have reported live shoot biomass values for mixed grass dominated grassland from 35 to 76 gm-2 at Pilani, India and 105 to 1974 gm-2 at Kurshetra (Singh and Yadava, 1974) , 24 to 457 gm-2 at Ujjain (Misra, 1973), 1 to 363 gm-2 at Ratlam (Billore, 1973) and 14 to 572gm-2 at Sagar for *Heteropogon* dominated grassland (Jain, 1971). *Glomus* species was the most commonly found species in all the plants. Similar results were reported by Beena *et al*. (2000); Bhuvaneswari (2010). *Acaulospora* sp. were also frequently observed. The hyphae of AM fungi play an important role in the formation and stability of soil aggregates and contribute to the composition of plant community structures (Smith and Read, 1997). Arbuscular mycorrhizal fungi have been described as'keystone mutualists' in ecosystems due to their unique position at the root-soil interface (Kumar *et al*., 2010). In this context the present research effort was made to understand the distribution, diversity, colonization rates, abundance and isolation frequency of AM fungi among the commonly available herbaceous plants from J. P. University Campus, Chapra. A total of 25 AMF morphotypes were recovered from 12 herbaceous plants which is considered to be a significant observation when compared to other previous observations of AMF association with plants (Rajkumar and Sunilkumar, 2011) and (Muthukumar *et al*., 2001). It is important to note that all the plants screened were mycorrhizal. Increased spore density did not show increased colonization rates in many herbaceous plants and vice versa. Based on RA and IF, *Glomus* and *Acaulospora* were the dominant genera and *Glomus fasiculatum, Glomus intraradices* and *Glomus claroideum* were the dominant species. Bever *et al*. (1996) reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment.

CONCLUSION

The present study confirms the occurrence of AM fungi in the herbaceous plants of J. P. University Campus, Chapra, Bihar. The live shoot biomass values in growing season is less than the other studies conducted in India. The communities formed in 2015, 2016 and 2017 were *D. annulatum-C. dactylon*. The mycorrhizal fungi have major impact on host plants even under any environmental condition and most important in the biodiversity. All the plant species studied were mycorrhizal; and the dominant genera were *Glomus* and *Acaulospora.* The most relatively abundance species was *Glomus intraradices* and the maximum isolation ferquency was recorded for *Glomus fasiculatum* .

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