Introduction
Leptadenia reticulata (Retz.) Wight and Arn. plant species are found in arid regions of Rajasthan (Kiran Bala et al., 1989; Pandey et al., 1999) which are characterized by poor sandy soils, low organic matter, uncertain and erratic rainfall, high wind velocity and generally experience water deficit during the growth period. Arbuscular mycorrhizal fungi are a major component of rhizosphere microflora in natural ecosystem and play significant role in the re establishment of nutrient cycling in native ecosystem (Peterson et al., 1985). The population of AMF varies greatly and their distribution is affected by various biotic and abiotic factors (Mohammad et al., 2003). Koske (1981) also reported that distribution of AM fungi is related to soil and environmental conditions. Hayman (1983) reported better establishment of vegetation in arid areas by using AMF as these fungi may often enhance plant absorption of phosphorus and other elements, improve water uptake and its transport to plants, and enable the plants to withstand high temperatures (Yaseen et al., 2011). Turnau and Haselwandter (2002) also considered AMF as a tool for re-establishment of endangered plant species. In the present investigation, in vitro raised Leptadenia reticulata (Retz.) Wight & Arn. with and without artificial mycorrhiza seeds were grown in the normal soil. The objective of this study is to determine the response of these seeds to plant survivability and growth.

Material and methods
The work was carried out at Department of Biotechnology, Poddar International College, Jaipur. The soil sampling of both the sites (site where the plant grows i.e. Jodhpur as well as the site where plant does not grow i.e. Jaipur, Fig: A) was done from Durgapura Agricultural Research Centre, Jaipur. The fungus was isolated from the soil in which the plant grows. This was done by spread- ing the soil on PDA plates and incubating the plates at 26° ± 2°C for growth. The isolated colony was identified by biochemical testing from S.P. Institute of Biotechnology, Jaipur. The fungi from the plate were spread onto a glass slide with the help of inoculation loop and stained with trypan blue and observed under microscope. The liquid culture of the isolated AMF was prepared by procuring the fungi from the culture plate and diluting it in 10 ml of water. Further the culture was kept for incubation at 25° ± 2°C for 48 hrs. Sodium alginate (3%) was mixed with liquid culture of AMF and the solution was singly dripped into a calcium chloride solution for a few seconds. The so-formed beads were kept on PDA plates for germination. The plates were kept at an incubation tempera-
supported in accordance to the finding. A positive correlation with organic carbon content in soil coincides with the findings of Mohammad et al., (2003), who reported the same result while investigating under semi-arid environment of Jor-
dan. Organic matter content in the soil increases the water-
holding capacity of the soil (Brady and Weil, 1996; Panwar and Tarafdar, 2005; Carrenhoet al., 2007) and, therefore, may facilitate a more favourable soil moisture condition for the AMF population. The phosphorus content (5.3 kg/hec) of the soil came out to be high as compared to Jodhpur soil (3.1 Kg/hec) which again suggested a lower population of AMF in Jaipur soil. The rhizosphere soils collected from field and successive pot cultures in Jodhpur have higher AMF spore densities compared to other sites (Panwar and Tarafdar, 2005). This may be because of poor soil fertility (in terms of available phosphorus) which results in higher AMF populations (No-
rani, 1996; Panwar and Tarafdar, 2005; Carrenho et al., 2007 ). In general, soils remained alkaline in reaction, became low in organic matter content and available phosphorus status.

The isolated fungus (Fig: B) was confirmed by S.P. Institute of Biotechnology, Jaipur. Mycelium and spore was mounted in lacto phenol for identification. The identification was based on spore and mycelium colour, size, surface ornamentation and wall structure with reference to the descriptions and pictures provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and originally published species descriptions (Fig: C-D).

The investigated sample is Scutellospora reticulata which is Arbuscular Mycorrhizal Fungi AMF was thus immobilized for the production of synthetic seed (Fig: E). Alginate was used as a matrix for the immobi-
ization of fungus culture. Alginate beads have been widely used for entrapment of BCAs like PGPR and fungi (Trivedi & Pandey, 2008), including AMF (Declerck et al., 1996; Vega et al., 2003). Vega et al., 2003 also worked on growth of micropropagated bananas colonized by root organ culture produced arbuscular mycorrhiza fungi entrapped in calcium alginate beads. Some findings was also reported on immo-
obilization of Pellets (2 mm) of the fungus T. Viride and their activity of β-glucosidase was investigated using cellbiose and salicin as substrates (Matteau and Saddler1982). Royer et al., (1983) also reported on hyphal outgrowth from beads that contained viable mycelial fragments. Immobilized fungi were first investigated for a role in biotransformations such as that of cortexalone to hydrocortisone by Curvularialunata (Sonomoto et al.,1983) and of glucose to itaconic acid by Aspergillus terreusn (Horitsu et al., 1983). Immobilized fungi are increasingly studied for application in processes which have traditionally only used free mycelia. The artificial seeds germinated on PDA media after 4-5 days (Fig: F).

The in vitro raised plant was grown on Jaipur soil (Fig: G) inoculated with artificial seeds as well as soil without the seeds. The growth in both the cases was recorded alternatively after 15 days. The growth of 6 cm was observed after 30 days (Fig: H) which was measured upto 15 cm after 90 days (Table-2) (Fig: I) in soil inoculated with artificial seeds while the plantlets raised on normal soil were unable to sustain (Fig: J). It led us to conclude that artificial seeds can be used to raise Leptadenia reticulata (Retz.)Wight and Am.in arid as well as semi arid regions with the help of artificial seeds. Positive ef-
effect of AMF beads on rice plant has ample of evidences (Seli-
cia& Bagyaraj, 1994; Xiao et al., 2010). Similar results were reported in cowpea (Vignaunguiculata) varieties where AM inoculated plants out performed than non-inoculated plants (control) in terms of growth, productivity parameters and nu-
trient uptake (Yaseen, et al., 2011). Finally in order to make a confirmatory check the roots of the in vitro raised plant was stained and observed at Department of Zoology, Jaipur. Cleared and stained roots showed the presence of vesicles, arbuscules and hyphae which confirmed the infection of in vitro raised plant with AMF. The fungi identified by (Fig: K-L).

### Table-1: Comparison of physicochemical characteristics of both site soils

| S. No | Region | pH  | Electrical conductivity (Ds/m) | Organic carbon (%) | Olsen P (Kg/hec) |
|-------|--------|-----|--------------------------------|--------------------|-----------------|
| 1.     | Jaipur | 8.1 | 0.9                           | 0.15               | 5.3             |
| 2.     | Jodhpur| 8.3 | 0.10                          | 0.19               | 3.1             |

### Table-2: Growth of the plant recorded.

| S. No | No. Of days | Length of the plant | Soil inoculated with artificial seeds | Normal soil |
|-------|-------------|---------------------|--------------------------------------|-------------|
| 1.     | 30          | 6 cm                | 2 cm                                 |             |
| 2.     | 60          | 10 cm               | 3 cm                                 |             |
| 3.     | 90          | 15 cm               | Not survived.                        |             |
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| 2      | 60          | 10 cm              | 3 cm                                |             |
| 3      | 90          | 15 cm              | Not survived                        |             |

Fig A: Soil of both the sites for sampling.

Fig B: Isolated fungi on PDA plate.

Fig C-D: Arbuscular fungi as identified after staining.

Fig E: Artificial seeds prepared by immobilizing AMF.

Fig F: Germinated synthetic seeds on PDA plate.

Fig G: In vitro raised plant grown on normal soil along with synthetic seeds.

Fig H: Growth of plant after 30 days (6 cm).

Fig I: Growth of plant after 90 days (15 cm).

Fig J: In vitro plant grown on normal soil along without synthetic seeds.
Fig K-L: Fungi infected roots of in vitro raised plant.

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