ABSTRACT
Total 110 seed samples of brinjal (Solanum melongena L.) were collected from 12 major growing districts of Rajasthan and subjected to dry seed examination (DSE). DSE revealed asymptomatic (07.75-97.5%), moderately discolored (04.50-67.50%) and shriveled discolored (03.25-38.75%) seeds. Symptomatic seeds in DSE showed various types of discolorations, deformation like white crust on seed surface. The seeds with water soaked symptoms and spots on seed surface were also observed such seeds on incubation yielded bacterial species. The incubation of symptomatic seeds about 21 fungal species of 14 genera and 3 bacterial species were observed viz. Alternaria alternata, A. solani, Aspergillus flavus, A. niger, Cladosporium oxysporium, Curvularia lunata, Fusarium oxysporium, F. solani, Rhizoctonia bataticola and Rhizopus nigricans etc and bacterial species like Xanthomonas axonopodis var. vesicatoria,Ralstonia solanacearum and Pseudomonas aeruginosa. The microflora severely affects seed germination (failure or delayed germination), wilting and rotting of seedlings, bacterial oozing, collapse of hypocotyls and cotyledonary leaves which resulting seedling mortality. High yield of microflora was obtained on standard blotter method (SBM) and agar plate method (APM).

Keywords- Brinjal Diseaes, Phytopathological Effects, Seed-Borne Microflora, Incidence of Microflora, Seed Sample Survey.

I. INTRODUCTION
Brinjal (Solanum melongena L.) is origin of Indo- Burma region belongs to family Solanaceae and commonly known as egg plant, aubergine, or guinea squash. It is a warm-season, non-tuberous, summer vegetable grown in tropical and temperate parts of the world [1]. Now the plant is widely cultivated in all over India, particularly in West Bengal, Orissa, Andhra Pradesh, Gujrat, Bihar, Madhya Pradesh, Maharashtra, Chhattisgarh, Karnataka and Haryana as major brinjal growing states. Rajasthan accounts brinjal for about 21803 MTs with an area of 4633 Ha [2]. Several diseases pose a great threat in cultivation of brinjal. These diseases not only reduce the yield but also deteriorate the quality of fruits. Fungal diseases are very much hazardous to brinjal plants in Rajasthan region because of temperature flux. Various types of diseases as leaf spot, leaf blight, root rot, fruit rot and post-harvest diseases found to be associated with brinjal. The seed to seedling transmission of seed-borne pathogens create alarming situation. The seed-borne fungi infect brinjal seedlings and cause severe losses due to damping-off, collar rot, stem canker, leaf blight, fruit rot that resulting in premature defoliation, less in number and size and quality of fruits up to 20-30% [3]. Screening of literature reveals that no planned and specific survey has been made as yet on the parasitic and storage diseases of plant in Rajasthan. During this investigation a number of fungi and bacterial species were found to be associated with the various plant parts. These infected fruits are short lived and greater loss is caused during transit and storage. The seeds are planting material for new generation but affected to various plant pathogens reduced to yield and spread of pathogens to next generation. The aim of present study is to investigate the major seed-borne disease, their effect on germination, yield loss and their incidence in context of Rajasthan.

II. MATERIALS AND METHODS
I. Study area and collection
The experiment was conducted in PG Department of Botany, Agarwal P.G. College, Jaipur (Rajasthan) in 2012 to 2016. The survey was conducted in 12 major brinjal seed growing district of Rajasthan. A total of 110 seed samples of brinjal were collected from different farmer field, storage houses and open market to know the diversity and incidence of microflora associated with them.

II. Detection of seed-borne pathogen
The seeds samples were subjected to Dry Seed Examination (DSE), Standard Blotter Method (SBM) and Agar Plate Method (APM) for detection of seed-borne pathogen. In dry seed examination, 100 seeds were taken randomly and examined by naked eye as well as by stereo binocular microscope (Nikon). In SBM, 100 untreated; 100 pretreated seeds (treated with available 2% aqueous sodium hypochlorite) were placed on water soaked blotter papers in Petri dishes. The Petri dishes containing seeds were incubated at 22± 2 °C under alternating cycles of 12 hours near ultraviolet (NUV) light and darkness for 7 days. Seed germination percentage and seedling symptoms were recorded on 8th day of incubation.
external and internal seed-borne fungi are identified or detected by two important commonly methods viz. Blotter method and Agar plate Method. In APM, the seeds were incubated on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) for isolation of fungi and bacteria, respectively [4,5]. Percentage of incidence and germination of mycoflora was calculated by following formula:

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\text{Incidence} \% = \frac{\text{No. of seeds infected to pathogens}}{\text{Total number of seeds observed}} \times 100
\]

(iii) Identification of Pathogen:

Growth of different type of fungal pathogen was examined under the steriobinocular microscope and identified by various characteristics, staining techniques etc.

III. RESULTS AND DISCUSSION

(i) Dry Seed Examination

The collected seed samples revealed asymptomatic (07.75-97.5%), moderately discolored (04.50-67.50%) and shriveled discolored (03.25-38.75%) seeds (Table 1). The seed samples have dark brown or black discolored seeds 57 (03.50-32.75%), seeds with fruiting bodies 18 (03.25-17.75%), white mycelial crusted seeds 49 (01.25-25.75%), brown spotted discolored seeds 51 (03.25-31.25%), distorted or shriveled seeds with white crust 42 (01.25-22.75%) and insect damaged or cracked seeds 37 (02.25-31.25%). The seeds having oozing, water soaked symptoms, shriveled and discolored seeds 110 (07.50-39.25%) (Table 2). The infected seeds with symptoms act as primary inoculums [6], Gupta et al. (1989) [7] observed discolorations on the surface of okra seeds at micropylar end caused by Alternaria alternata, Drechslera sp., Curvularia lunata and Aspergillus spp. Brinjal seeds in storage infected by Aspergillus flavus, A. niger, Fusarium moniliforme and Helminthosporium tetramera [8].

(ii) Incubation Test

A total of 21 fungal species of 14 genera were yielded on SBM. Among them Alternaria alternata (06-33%), A. solani (05-31%), Aspergillus flavus (03-40%), A. niger (02-36%) Curvularia lunata (03-21%), Fusarium oxysporum (03-27%), F. solani (03-31%), Rhizoctonia bataticola (05-19%) and Rhizopus nigricans (05-27%) were found dominant fungi. Chlorine pretreated seeds reduces the incidence of saprophytic fungi and enhances seed germination (Table-2 and Table-3).

(iii) Incidence on agar plate method (APM)

Fusarium oxysporum, F. solani, Aspergillus flavus, A. niger, Curvularia lunata, Alternaria solani and Rhizoctonia bataticola had high per cent incidence on PDA media. Except the fungi reported on SBM there are a few fungi named as Aspergillus terreus, Rhizoctonia solani, F. pallidoroseum, Trichoderma roseum, T. harzianum, Mennoniiella echinata and Chaetomium murorum were also found on PDA. The bacterial species Pseudomonas syringae pv. syringae, Xanthomonas campestris pv. campestris and Erwinia caratovora were also reported on nutrient agar media (NA) with low incidence.

(iv) Germination percentage

Poor germination was noticed in stored seeds depends on period of storage. The seedlings yield from infected seeds was found stunted and died prematurely infected with F. moniliforme and F. solani. Seeds infected with various species of Aspergillus showed decline in germination to an extent of 45%. Whereas seeds infected with Alternaria alternata, A. solani and Curvularia lunata reduce germination up to 39%. Similar results have been reported on brinjal seeds infected by Alternaria solani [9,10] and A. porri.

(v) Phytopathological Effects

The microflora associated with brinjal seeds caused adverse effect to seed germination and produce various symptoms on seedlings. Seeds germination was found improved after treatment in SBM. The shriveled or ungerminated seeds have oozing and rotting on SBM or such seeds showed poor and delayed germination. In this study it was observed that the germination was hampered by the species of Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Fusarium, Penicillium, Rhizopus and Rhizoctonia. Alternaria alternata, A. solani and Rhizoctonia bataticola produced brown black streaks on hypocotyls, browning of radicle which later on at severe infection the whole seedling collapses. Various species of Aspergillus caused brown to black lesions on hypocotyls and browning of radicle. Infection of species of Fusarium showed yellowing, dying and wilting of seedlings.

Alternaria alternata causes shriveling, discolorations in seeds, reduced seed germination and seedling blight in other crops [11, 12]. Fruit rot in brinjal is caused by Alternaria alternata [13] and Rhizopus nodosus [14]. Wilt and fruit rot caused by various species of Fusarium [15], F. oxysporum f.sp. melongenae [16], Verticillium dahliae [17], Phomopsis, Alternaria, Colletotrichum, Fusarium, Rhizopus and Cercospora [18] and Phoma spp, Botryodiplodia theobromae, Rhizopus spp., Absidia sp. and Fusarium spp. [19].

Mehta and Mehta (1989) [20] found that fruit rot Trichosanthes dioica and Solanum melongena caused by Fusarium oxysporum and F. moniliforme respectively. Macrophomina phaseolina was found the most pathogenic in germination trials produce both sclerotia and pycnidia on seed surface that predominantly reduce the seed quality in cucurbits [21] and chili [22]. Black dot root rot of eggplant caused by Colletotrichum atramentarium [23], leaf spot disease Colletorichum dematium [24] produced symptoms on leaves Erysiphe polyphaga [25] with whitish powdery areas on both the leaf surfaces. Seeds with white crust, discolorations and water soaked symptoms yielded Actinomyces spp., Xanthomonas axonopodis pv. vesicatoria, Ralstonia solanacearum and Pseudomonas aeruginosa in this
study. Such symptoms were also observed in chilli, tomato [12], pea [26], okra [27] and many other crops.

### IV. CONCLUSION

In this study it is evident that samples collected from different parts of Rajasthan had considerable microflora. The percent incidence of the fungi was higher in the blotter test. The seed samples had poor germination due to storage. It is general practice in some areas that ripe fruits are dried in sunlight on the soil before collecting the seeds. This practice may lead to contamination of the seeds by soil containing fungi. Species of *Fusarium*, *Aspergillus* produce toxins which may hasten the reduction in germination. At the time of seed collection from field the seed sample contaminated with debris promote the growth of fruit rot pathogens. Proper seed cleaning and seed dressing is essential to avoid losses in storage and control of seed-borne fungi.

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### Table 1: Disease incidence occurs in dry seed examination (DSE) in brinjal seed grown in different locations of Rajasthan

| S. No. | Districts | Total number of seed samples | Number of seed samples infected |
|--------|-----------|------------------------------|-------------------------------|
| 1.     | Jaipur    | 51                           | 51 (03.25-38.75)              |
| 2.     | Dausa     | 6                            | 06 (12.25-25.50)              |
| 3.     | Tonk      | 8                            | 08 (06.75-14.50)              |
| 4.     | Jhunjhunu | 5                            | 05 (05.25-13.75)              |
| 5.     | Kota      | 7                            | 07 (08.75-19.25)              |
| 6.     | Alwar     | 2                            | 02 (08.25-10.75)              |
| 7.     | Sikar     | 8                            | 08 (05.25-34.75)              |
| 8.     | Jalore    | 5                            | 05 (06.25-21.75)              |
| 9.     | Nagaur    | 3                            | 03 (07.25-18.00)              |
| 10.    | Bikaner   | 8                            | 08 (08.75-29.75)              |
| 11.    | Jodhpur   | 2                            | 02 (11.25, 17.50)             |
| 12.    | Ajmer     | 5                            | 05 (09.25-21.75)              |
| **Total** | **110**    | **110 (03.25-38.75)**        |                              |

### Table 2: Changes in seed morphology, percent incidence of pathogens associated with brinjal seeds

| S. No | Type of seed discoloration | Occurrence | Incidence (RPO) | Microorganism associated with seeds                                    |
|-------|----------------------------|------------|----------------|------------------------------------------------------------------------|
| 1.    | Brown or black discoloured  | 57         | 03.50-32.75%   | *Curvularia* spp., *Drechslera* spp. and *Alternaria* spp.             |
| 2.    | Seed with fruiting bodies  | 18         | 03.25-17.75%   | *R. bataticola*, *Aspergillus* spp                                     |
| 3.    | White mycelial crusted seeds| 49         | 01.25-25.75%   | *Fusarium* oxysporum, *F. moniliforme*, Actinomyeetes spp.             |
| 4.    | Brown spotted discoloured  | 51         | 03.25-31.25%   | *Alternaria* spp., *Curvularia* spp., *Chaetomium* and *Myrothecium* spp. |
| 5.    | Distorted or shrivelled seeds with white crust | 42 | 01.25-22.75% | *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp.              |
| 6.    | Insect damaged or cracked seeds | 37 | 02.25-31.25% | *Aspergillus* spp., *Chaetomium* spp., *Penicillium* spp., *Rhizopus* spp., insects and... |
7. Seeds with oozing, white crust, water soaked, shrivelled and discoloured 110 07.50-39.25% *Ralstonia solanacearum*, *Xanthomonas axonopodis* pv. *vesicatoria*, *Pseudomonas aeruginosa*

| S. No. | Fungi                        | Untreated | Pre-treated |
|-------|------------------------------|-----------|-------------|
|       | Occurrence | RPO | Range (%) | Occurrence | RPO | Range (%) | Phytopathological effects |
| 1     | Actinomycetes                | 21 | 19.09     | 01-15% | 08 | 07.27     | 1-7% | Delayed germination |
| 2     | Alternaria alternate         | 37 | 33.63     | 06.63% | 33 | 30.00     | 2-20% | Leaf blight and leaf spot, symptoms on hypocotyls and cotyledons |
| 3     | A. solani                   | 33 | 30.00     | 05-31% | 23 | 20.90     | 4-23% |
| 4     | Arthrobotrys superbus        | 17 | 15.45     | 02-13% | 12 | 10.90     | 1-8% | Seed rotting, discolourations |
| 5     | Aspergillus flavus           | 43 | 39.09     | 03-40% | 32 | 29.09     | 2-25% |
| 6     | Aspergillus niger            | 39 | 35.45     | 02-36% | 33 | 30.00     | 2-26% |
| 7     | Aspergillus fumigates        | 13 | 11.81     | 03-9%  | 07 | 06.36     | 1-3% |
| 8     | A. candidus                  | 09 | 08.18     | 03-7%  | 04 | 03.63     | 1-3% |
| 9     | Chaetomium globosum          | 17 | 15.45     | 03-12% | 09 | 08.18     | 1-7% | Seed rotting, check the seed growth |
| 10    | C. spinosum                  | 11 | 10.00     | 01-10% | 06 | 05.45     | 1-6% |
| 11    | Cladosporium oxysporiun      | 25 | 22.72     | 03-21% | 13 | 11.81     | 2-17% | Seedling rotting and seedling blight |
| 12    | Colletotrichum dematium      | 15 | 13.63     | 01-12% | 09 | 08.18     | 1-6% | Fruit rooting and seedling rot |
| 13    | Curvularia lunata            | 25 | 23.63     | 03-21% | 17 | 15.45     | 1-13% | Leaf spot and rotting |
| 14    | C. intermedia                | 12 | 10.90     | 04-10% | 07 | 06.36     | 1-5% | Leaf spot and rotting |
| 15    | Fusarium equiseti            | 11 | 10.00     | 02-8%  | 08 | 07.27     | 1-4% |
| 16    | F. semitectum                | 09 | 08.18     | 01-5%  | 06 | 05.45     | 1-3% | Leaf spot and rotting die back, wilting and damping off |
| 17    | F. moniliforme               | 39 | 32.72     | 04-30% | 28 | 25.45     | 1-27% |
| 18    | F. oxysporum                 | 31 | 28.18     | 03-27% | 24 | 21.18     | 2-13% |
| 19    | F. solani                    | 37 | 33.63     | 03-31% | 29 | 26.36     | 1-16% |
| 20    | Penicillium chrysogenum      | 23 | 20.90     | 03-15% | 13 | 11.81     | 1-6% | Hampered the seed germination, Seedling rot |
| 21    | Rhizoctonia bataticola       | 25 | 22.72     | 05-19% | 18 | 16.36     | 2-13% | Seed rot, blackening of radical and hypocotyls, stunted seedling |
| 22    | Rhizopus nigricans           | 32 | 29.09     | 05-27% | 13 | 11.81     | 1-11% | Collar rot, root rot and seed rot |

*Table 3: Different percent range of microflora in untreated and pre-treated seeds and their phytopathological effects*
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