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Phytotoxicity of Culture Filtrate of *Rhizoctonia bataticola* on Viability, Germination and Seedling Growth of Okra Seeds

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Abstract **:** In present study, seeds of five cultivars of okra *viz.* Arka Anamika, Pusa Sawani, Arka Veena, Jai Jawan-Jai Kisan and Parbhani Kranti were used to study the toxicity of culture filtrate (CF) of *Rhizoctonia bataticola* on viability, germination & seedling growth of okra. The viability of excised embryos was assayed by Tetrazolium Chloride test. It was decreased by 4, 16 and 24 h treatments with CF respectively. Complete loss of viability was observed in 30 h CF treated embryos as no staining took place. Embryos with 16 h of CF treatment showed a decrease in viability of seeds in all 5cultivars. Seeds of all cultivars soaked in distilled water for one day and treated separately for 24 h with CF. In CF treated seeds of all cultivars, germination was decreased 42, 58, 46, 69 and 40 % as compared to the control (non CF treated seeds) 70, 76, 82, 91 and 80 % respectively on 8th day of incubation. Maximum symptomatic seedlings (radical browning & brown- black streaks on hypocotyls) and seedling mortality were observed in Arka Anamika (83.33%, 45.71 %) and Prabhani Kranti (70%, 46.42 %) as compared to control of each cultivar (2, 2% respectively). CF treatment of pathogen caused severe toxic effect on seedling growth of already germinated seeds of all 5 cultivars.

Index Term**: CF *R.bataticola*, seed viability, seed germination, seedling growth, okra cvs.**

INTRODUCTION

Okra (*Abelmoschus esculentus* L.Moench) known as Lady Finger/ Bhindi is an important vegetable crop in India. It is mainly grown in Ajmer, Alwar, Bharatpur, Bundi, Chittorgarh, Dausa, Jaipur, Sriganganagar, Sirohi,Tonk and Udaipur districts of Rajasthan. Okra seeds have been proposed as a new source of protein for human nutrition (Karakoltsidis and Constantinides,1975). The crop okra losses its quality and yield due to infection caused by many fungal diseases (Singh, Bisht and Majumdar, 1988). A seed -borne disease caused by *Rhizoctonia bataticola* (Taub.) Butler syn.of *Macrophomina phaseolina* (Tassi) Goid in okra reported by Richardson(1990) and root and collar rot disease in okra by Rao and Mukerji(1972a)& Goel and Mehrotra(1973). Agrawal and Singh (2000) reported transmission of the disease due to extra-and intra embryonal infection of *M .phaseolina* in okra seeds.

MATERIAL AND METHODS

Pure culture of *R. bataticola* was prepared by 3 day old growth after incubation at 25^{0}C for 30 days (Fig: 3-A). The culture was filtered through whatman filter paper no.42 and centrifuged for 20 min at 3000 rpm.The autoclaved supernatant solution, the culture filtrate (CF) was assayed for its effect on viability, germination and seedling growth of 5 okra cultivars seeds.

(A) Effect of CF on seed viability using TTC Test.

Okra seeds of all cvs. were softened by soaking in distilled water separately for 4-5h after that seed coat and endosperm were removed. Excised embryos were kept into the CF for period of 4, 16, 24 and 30h.Seeds treated with distilled water for the same time period were considered as control. The treated embryos were blotter dried and kept in 0.1% solution of 2, 3, 5-triphenyl tetrazolium chloride GR (TTC) at 30^{0}C in dark. Staining of embryos was observed every 2h intervals up to 6h.

(B) Effect of CF on Seed Germination and Seedling Growth

Four replicates of 100 seeds of each cultivar were surface disinfected and soaked separately in CF for 24 h. For effect on seedling growth presoaked (24 h in distilled water) seeds were treated with CF for 24 h. 10 seeds/petrie plates were incubated on moistened blotters. Data were taken on germination, symptomatic seedlings, seedling mortality on 8th day of incubation. For control, seeds treated with sterilized water were used.

RESULTS AND DISCUSSION

Effect of Culture Filtrate of *Rhizoctonia bataticola*

(A) On Seed Viability of 5 okra cvs.

Tetrazolium chloride test was applied to check the viability of embryos by the production of pink –red color in emryonal tissues. The cotyledon and embryonic axis of viable seeds were completely stained (Fig:4 B). The seed viability decreased by 4, 16 and 24 h treatments with CF in all 5 cultivars as compared to control (70, 70, 70, 70 and 60 %) respectively (Fig:1). The prolonged treatment of 30 h with CF caused complete loss of viability and embryos were not stained whereas 16 and 24h CF treated embryos showed varied degree of staining pattern and thus loss in viability in all the 5 cultivars. Arka Anamika and

Parbhani Kranti were highly affected by the infection of CF of *R. bataticola* whereas other varieties were found moderately resistant. Grover and Singh (1970) reported similar varietal difference with regards to penetration of *F. oxysporum* in the host plants. Ibraheem, Okesha and Mhathem (1987) have reported decrease in rate of seed germination with increase in time period of CF treatment of *Alternaria alternata* from 1-24 h in soybean seeds. Godika (1995) also reported a decrease in viability and germination of seeds and seedling growth in sunflower by the CF of *R. bataticola* and *Alternaria alternata.* Toxic and inhibitory effect of CF of *Macrophomina phaseolina, Aspergillus flavus, A.niger, A.terreus, Fusarium. semitectum, Chaetomium* sp. on seed germination of cowpea, okra and brinjal was also reported by Dash and Narain (1996a).

(B) On Seed Germination and Seedling Growth of 5 cvs. of okra

CF treatment caused severe toxic effect on seedling growth of already germinated seeds in all 5 cultivars. In CF treated seeds, **seed germination** was decreased (42, 58, 46, 69 and 40 %) as compared to their control (non CF treated seeds) 70,76,82,91 and 80% respectively in all cvs. of okra on 8th of incubation.. Symptomatic seedlings showed symptoms as radical browning and streaks on hypocotyls (Fig:4D) were observed maximum in Arka Anamika (83.33%) and Parbhani Kranti (70%) as compared to control of each cultivar (2, 2% respectively). In Arka Veena, Pusa Sawani and Jai Jawan –Jai Kisan infected seedlings from CF treated seeds ranges from 39.19 % to 17.39 % (Table.1). Seedling mortality was observed in cv. Jai Jawan- Jai Kisan (58.33%) followed by Prabhani Kranti (46.42%) and Arka Anamika (45.71%) after 8 th day of incubation (Table-1).

For the effect of CF of *R.bataticola* on **seedling growth**, seeds of 5 cvs. were soaked in distilled water for one day and treated separately for 24h with culture filtrate. The CF treatment caused drastic adverse effect on growth of seedlings (Fig:E-G). Cultivar Arka Anamika and Parbhani Kranti showed 100% symptomatic seedlings followed by Jai Jawan-Jai Kisan (80%), Pusa Sawani (57.69%) and Arka Veena (56.25%). On 8th day of incubation, seedling mortality was high in Parbhani Kranti (88%) followed by Arka Veena (83.33%) and Jai Jawan-Jai Kisan (66.66%) respectively (Table-2) Culture filtrate of *Curvularia lunata, F.moniliformae, Aspergillus* sp., *Cladosporium* sp., control the germination of seeds and root –shoot length reported by Subbaraja (1973). Toxins of the seed-borne fungi are also known to reduce the seed germination and root- shoot growth in Soybean (Anahosur and Bidari, 1974) Sorghum (Anahosur, 1976). Prasad and Prasad (1980) observed a marked adverse effect of CFs of *A.flavus, A.niger, Fusarium lini, Penicillium chrysogenum, Chaetomium globosum, Trichoderma viride, Curvularia lunata* and *Alternaria lini* on percent seed germination and seedling growth of *Linum usitatissimum.* Bavaji, Latheef and Sreeramulu (2000) were also reported reducing effect on seed germination and seedling growth of *Sesamum indicum* with CF of *Alternaria alternata* (7, 15, 30, 45 and 60 day old culture filtrate). Jalander and Gachander (2012) reported a decrease in seed germination and seedling growth in cowpea due to toxicity of culture filtrate of *Aspergillus* species. Goel and Mehrotra (1974c) reported that cellulolytic and pectolytic enzymes in culture filtrate of *R. bataticola (M.phaseolina*) play a vital role in causing browning and maceration of tissues. Chhabra, Sidhu and Singh (1977) observed a decrease in percent seed germination, root-shoot length, wet and dry weights of 7 day old okra plants which were inoculated with culture of *Meloidogyne incognita* and *R. solani* simultaneously. In present study, culture filtrate of *Rhizoctonia bataticola* contains some mycotoxic substances which caused pathogenesis and inhibitory effect on viability, germination of okra seeds and their growth of seedlings.

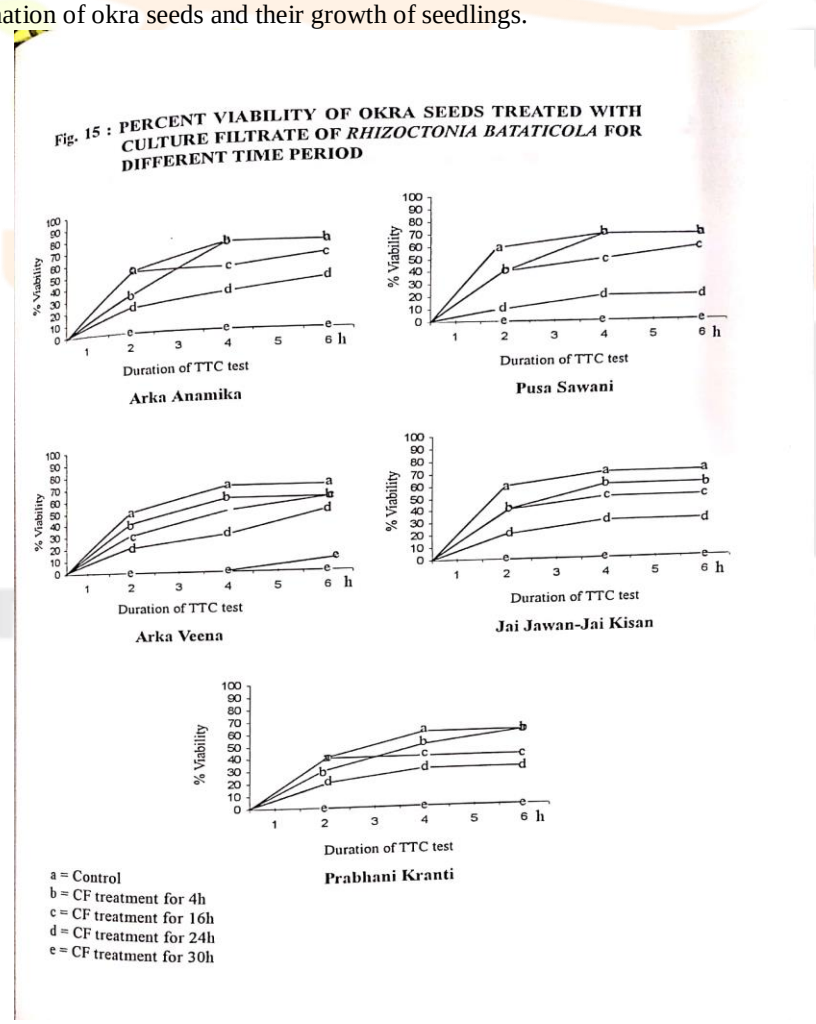


Fig: 1- Percent viability of okra seeds (cvs:Arka Anamika, Pusa sawni, Arka Veena,Jai Jawan-Jai Kisan and Parbhani Kranti)treated with CF of *Rhizoctonia bataticola* for different time period.

Table 1: Percent seed germination of okra seeds treated with Culture Filtrate of *Rhizoctonia bataticola* (100 Seeds/cv)

Cultivars	Germination% (Control-untreated)	Germination% (CF Treated)	Symptomatic Seedlings%	Seedling Mortality%
Arka Anamika	70	42	83.33	45.71
Arka Veena	82	46	39.13	16.66
Jai Jawan-Jai kisan	91	69	17.39	58.33
Parbhani Kranti	80	40	70	46.42
Pusa Sawani	76	58	34.48	30

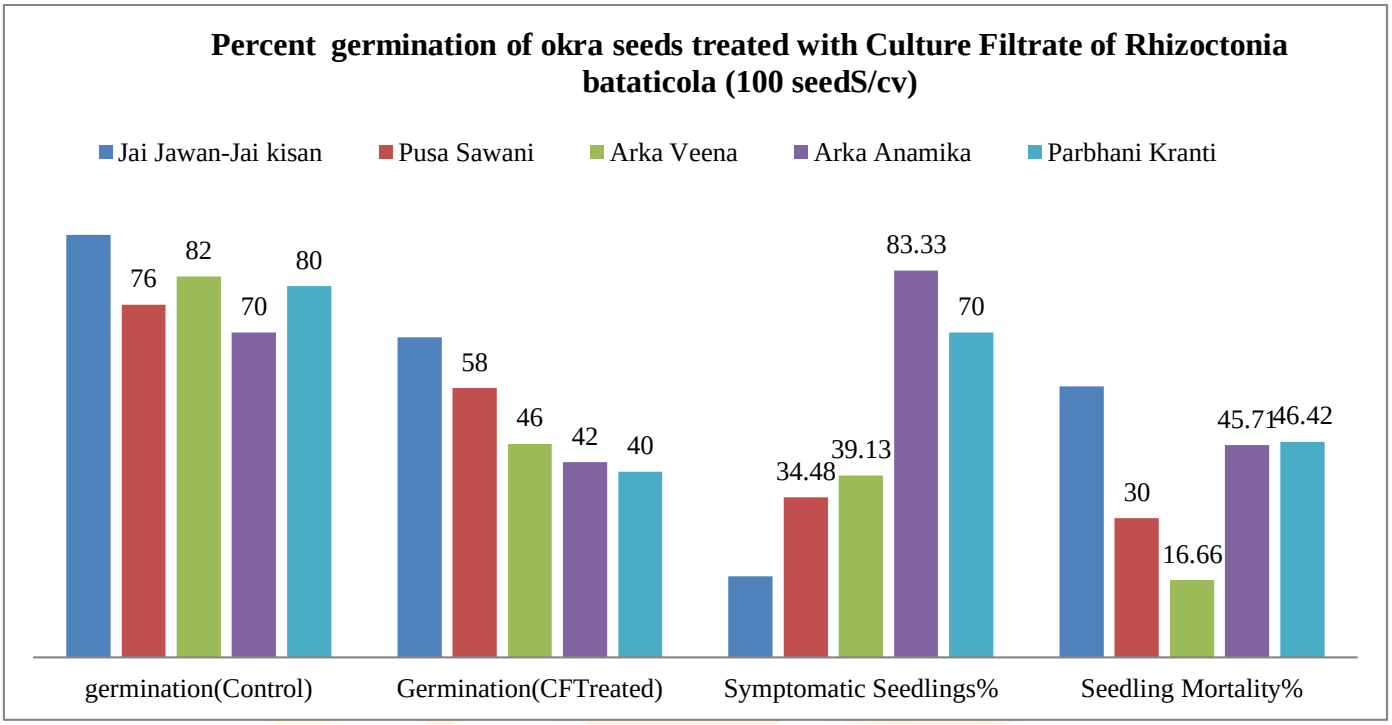
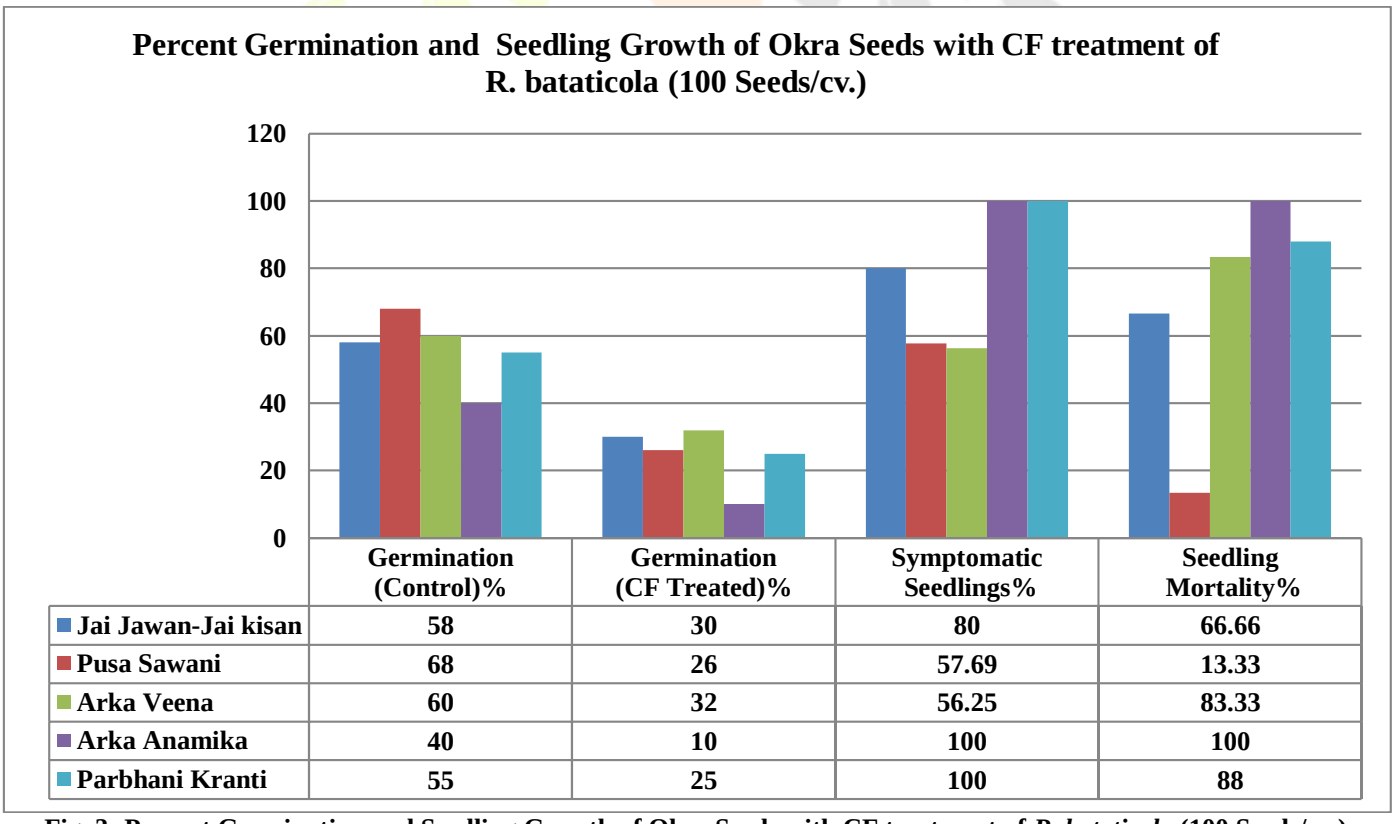


Fig.2: Effect of culture filtrate of *R. bataticola* on germination of 5 cultivars of okra seeds.



	Germination (Control)%	Germination (CF Treated)%	Symptomatic Seedlings%	Seedling Mortality%
Jai Jawan-Jai kisan	58	30	80	66.66
Pusa Sawani	68	26	57.69	13.33
Arka Veena	60	32	56.25	83.33
Arka Anamika	40	10	100	100
Parbhani Kranti	55	25	100	88

Fig. 3: Percent Germination and Seedling Growth of Okra Seeds with CF treatment of *R. bataticola* (100 Seeds/cv.)

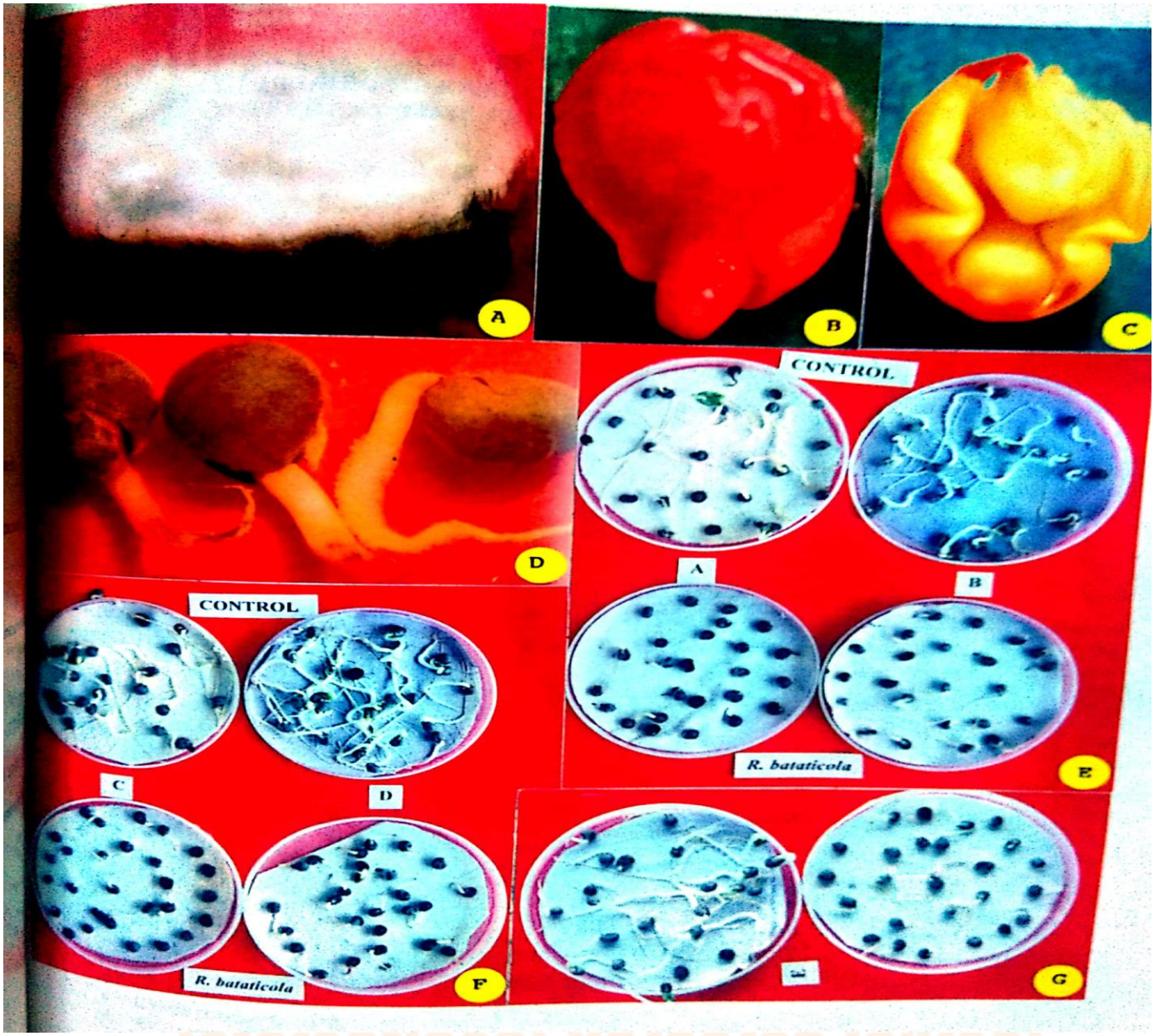


Fig. 4
(A) Growth of pathogen on liquid Richard's medium after 30 days.
(B). Non CF treated seeds (control) showing viability
(C) Unstained CF treated seeds remain non viable X 18,X 12.
(D) .Healthy seedling (right) and 24 h CF-treated seed after incubation showing rotting of radical (middle and left) X 12.
(E-G) .Seeds of okra cultivars *viz*., (A) Arka Anamika, (B) Pusa Sawani, (C) Arka Veena, (D) Jai Jawan-Jai Kisan and (E) Parbhani Kranti treated with CF for 24hand after incubation showing loss in seed germination, symptomatic seedlings in comparison to their control.

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