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ISOLATION OF FUNGAL PATHOGENS FROM SEEDS OF SELECTED VEGETABLES AND STUDY OF THEIR IMPACT ON GERMINATION AND SEEDLING GROWTH

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ABSTRACT

In the present study seeds of 5 vegetables such as spinach (*Beta vulgaris* L.), Bhindi (*Abelmoschus esculentus* L.), cucumber (*Cucumis sativa* L.), tomato (*Lycopersicon esculentum* L.) and egg plant (*Solanum melongena* L.), were obtained from the stored stocks of the trader. Altogether ten fungal pathogens viz., *Alternaria* spp., *Curvularia* spp., *Fusarium* spp, *Penicillium* spp., *Colletotrichum* spp., *Aspergillus* flavus, *Aspergillus niger*, *Macrophomina* spp., *Phomopsis* and *Cladosporium* were isolated from the aforesaid seeds of vegetables. Four fungal pathogens were isolated form spinach, six from bhindi, four from cucumber, four from tomato, and five from the seeds of egg plant. Highest percentage of germination 88 was recorded in cucumber, followed by spinach 80. Similarly, lowest percentage of germination of seeds 50 was found in bhindi. Maximum percentage of failure in germination was found in bhindi and lowest 12 in cucumber. Higher percentage of normal seedlings 78.0 was observed in cucumber followed by egg plant that was 76.0. Minimum percentage of normal seedlings was recorded in bhindi that was 47 only. The percentage of abnormal seedlings was 4 in bhindi, cucumber and tomato respectively. It was 3% in both spinach and egg plant. Highest diseased seedlings 4, was found in tomato and lowest 2 in spinach. Maximum number of dead seedlings was found in bhindi, which were 46 on 7th days, 47 on 14th and 54 on 21st day of sowing. Lowest number of dead seedlings was found in case of egg plant, for all the stages of observations. Mean shoots and roots lengths were also measured. It was highest shoot length 16.30 cm and roots 7.5 on 30th day of sowing. Highest vigor index 1956.0 was noted in cucumber, while the lowest shoot length 3.80, root length 2.50 and vigor index 469.35 were found in case of egg plant.

KEYWORDS: Seed Borne Pathogen, Normal Seedling, Abnormal Seedling, Diseased Seedling, Dead Seedling, Vigor Index, Germination Failure

As a leading producer of low-cost fruits and vegetables, India had an enormous export market. India ranks second in fruits and vegetable production in the world after China. The vast production base offers India tremendous opportunity for export. During 2019-2020, India exported fruits and vegetable worth Rs 9,182.88 crores/1,277.30 USD millions, which comprised of fruits worth Rs. 4,832.81 crores/668.75 USD million and vegetables worth Rs. 4350.13 crores/ 608.48 USD millions. It is estimated that between 30 to 35% of India's total vegetable is lost due to fungal diseases. (Rahul *et al*; 2015). Pathogen free seeds are considered as the vital factor for desired plant population and good harvest. Infection of seeds by fungal pathogens and presence of propagules of the fungal pathogens in a seed lot is vitally important because infected seeds or seed lot may fail to germinate, may cause infection to seedlings and growing healthy plants. Therefore, healthy seeds are considered as an important factor for successful crop protection. The lack of high quality seeds and the prevalence of seed borne fungal pathogens are the main constraints in getting the desired populations and production of the crops. About 20% of the known plant viruses are transmitted through seeds of infected plants (Agarwal and Sinclair; 1997). Various kinds of direct and indirect losses caused by plant diseases include reduced quality and quantity of crop produce, increased cost of production; threat to animal health and environment, limiting the type of crops/ varieties grown. In order to combat the losses caused by the plant diseases, it is mandatory to define the problem and seek solutions.

At the biological level, the requirements are for fast and accurate identification of the causal organism; accurate estimation of the severity of disease and its effect on yield, and identification of its virulence mechanisms. Disease may then be minimized by the reduction of the pathogens inoculums, inhibition of the virulence mechanism, and promotion of genetic diversity in the crop.

Current emphasis for increased crop yields is to increase seed replacement rate. This requires seed health and rigorous seed health testing. Seed pathology involves the study and management of disease affecting seed production and utilization as well as management practices applied to seeds. Research innovations in detection of seed borne pathogens and elucidations of their epidemiology, advances in development and use of seed treatments; and progress towards standardization of phytosanitory regulations are to be strengthened (Chahal, 2012). With the globalization of agriculture, seed health testing is going to be mandatory for seed quality control. Form the survey of literatures it was gathered that seed borne fungal pathogen causes severe damage to crop yields starting from seed germination, seedlings abnormalities, seedling mortality etc. All these can affect the desired yields of our corps in general and vegetables in particulars. Some of the references are being

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mentioned here. Agarwal *et al*; (1972); Rout and Rath (1974); Gangopadhyay and Kapoor (1975); Agarwal (1976); Kirkpatrick and Bazzaz (1979); Mathur (1983); Basak *et al*; (1989); Sati *et al*; (1989); Mathur (1995); Parveen and Ghaffar (1995); Agarwal and Sinclair (1997); Namesh (1998); Pushpa *et al*; (1999); Alimova *et al*; (2002); Natsugah *et al*; (2004); Chaudhary *et al*; (2005); Javaid *et al*; (2005); Nishi kawa *et al*; (2006); Pandey (2010), all have studied the mycoflora associated with seeds of different crops including vegetables. Srivastva *et al*; (2011) studied occurrence of fungal pathogens on brassicaceous vegetables crops and their control measures. Nation *et al*; (2011) reported seed mycoflora of tomato cultivar, collected from different localities of Gujarat.

Mandal *et al*; (2013); Sharma *et al*; (2013); all have worked with the seed mycoflora of different crops. Barry (2014) at the international workshop on seed borne disease of vegetables discussed the types of diseases and their control measures. Hamim *et al*; (2014) worked in detail about the types of fungal pathogens on different vegetable crops and their impact on the seed germination. Rahul *et al*; (2015) reviewed on post-harvest management of fungal diseases in fruits and vegetables. Dalfia and Valkonon (2016) reported fungal disease of common bean of Nicaragua. Mancini *et al*; (2016) discussed diagnostic methods for detecting fungal pathogens on vegetable seeds. Chaudhary *et al*; (2017) reported seed borne fungal pathogens associated with pigeon pea seeds. Chohan *et al*; (2017) reported management of seed borne fungal disease of tomato. Keeping all these ideas in mind present work was taken to isolate fungal pathogens from seeds of common vegetables of this locality and observe its impact on seed germination and seedling growth etc.

MATERIALS AND METHODS

Present work was done in the following sequences.

Collection of Seed Samples

Seeds of five vegetables as mentioned above were procured from the authorized seed merchant at Hajipur, of Vaishali district. All seeds were brought in the laboratory and kept in the well cleaned and dried plastic bottle having suitable caps. They were labeled and stored at low temperature, till the seeds were used for the subsequent experiments.

Detection of Fungal Flora of the Selected Vegetable Seed

For this well cleaned pre-sterilized, Petri plates with 9 cm diameter were taken. Blotting papers (Whatman No.-1) two in each Petri plates were lined properly after soaking in sterilized distilled water. In one plate, 20-25 seeds of spinach were placed. Three such plates were used for it.

Similar treatment was given for the seeds of bhindi, tomato and brinjal. However, only 10-12 seeds of cucumber were placed in fresh Petri plates lined with moist filter papers. All the plates were incubated in culture room. The light and dark periods were adjusted 12:12 and temperature was maintained at 22 ± 1^0C. Seeds were observed on an alternate day. The filter paper was kept moistened by spraying pre-sterilized distilled water. After seven days of incubation plates were observed under dissecting microscope. Fungal growth was found. For identification, spores with mycelium were selected with the help of sterilized needle under dissecting microscope.

They were mounted in Lactophenol containing cotton blue, on a well cleaned glass slide. A cover slip was placed and then detail study was done under research microscopes. Different species of fungus was identified on the basis of spores and other characters, with the help of standard books of Mycology and with the help of faculty member.

Germination Test

For determining the impact of pathogens on the germinations of seeds, seedling growth, abnormal seedlings, experiments were performed in the laboratory. For this sands were washed properly and dried. Then it was sterilized with 3% formaldehyde. Here for one Cft, 200 ml formaline was mixed properly. The sand was covered with polythene sheet for 24 hours. After this the above sheet was removed and sand was spread over a well cleaned polythene sheet. The sand was thus placed in oven for 7-8 days so that the formaldehyde gas was evaporated completely.

In the plastic tray above sand was spread and different, seeds of vegetables were sown separately. Before sowing the seeds, the trays were moistened with sterilized water. For each vegetable, three trays were used. After sowing the seeds of vegetables in different trays, the sand was kept moistened by spraying sterilized water. Observation was made on alternate day.

Observation was made for the germination of seeds, seedling growth abnormal seedlings, diseased seedlings, dead seedlings. All these parameters were noted on 7 days, 14 days and 21 days after showing. The shoot and root length was measured after uprooting the seedling with care. The sand particles were removed after proper washing. Based on the above, shoot and root length and number of seedlings the vigor index was determined by the following formula. Here 10 seedlings per replica were selected randomly. The mean of shoot and root length was taken for data analysis.

Vigor index = (Mean length of shoot + Mean length of root) X % seed germination

RESULTS AND DISCUSSION

In the present study, seeds of five common vegetables were procured form the authorized seed traders. The number and fungal species isolated from different vegetable seed was tabulated in table-1. From the table, it may be noted that form the seeds of spinach, altogether four fungal species such as *Alternaria* spp., *Curvularia* spp., *Fusarium* spp., and *Penicillium* species were isolated and identified. Here total number was 81.0. From the seeds of bhindi, six fungal species viz., *Fusarium* spp., *Penicilium* spp., *Aspergillus flavus*, *Aspergillus niger*, and *Macrophomina* spp. were isolated. Here the total number of all fungal species was 118.0. From the seeds of Cucumber, four fungal species such as, *Alternaria* spp., *Curvularia* spp., *Fusarium* spp,. and *Penicillium* spp. were isolated and identified. Here total number of fungal spp. was 62.0. From the seeds of tomato, also four fungal species such as *Fusarium* spp., *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium* spp., were isolated and identified. Here the total number was 82.0. On the seeds of egg plants altogether five fungal species such as *Curvularia* spp., *Fusarium* spp., *Aspergillus flavus*, *Aspergillus niger* and *Phomopsis* species were isolated. The total number of fungal species was 77.0.

From the table, it may be noted that all the fungal pathogens isolated from the same seeds were not equal in number. In case of spinach, *Fusarium* spp was in highest number that was 24.0 followed by *Alternaria* spp. that was 23.0. It may be noted that lowest number 15.0 was noted here for the fungal species *Curvularia*. In case of bhindi, highest number 25.0 was for *Aspergillus flavus*, followed by *Fusarium* spp. that was 22.0. Here lowest number, 14.0 was noted for *Penicillium* spp. In case of cucumber highest number 21.0 was found for *Fusarium* spp., while the lowest was noted for *Penicillium* sp. that was 10.0 only. In case of tomato also, *Fusarium* spp. had the highest number that was 25.0 followed by *Caldosporium* spp. which was 21.0. In case of egg plant seeds also the highest number 20.0 was for *Fusarium* and lowest 11.0 for *Aspergillus niger*. If we consider the frequency of the fungal species isolated and identified here, *Fusarium* spp. had the highest frequency as it was found in all the five seeds studied here. Presence of different fungal species on different seeds may be correlated with the physiological specialization of the species. It may further be noted that prevalence of seed borne fungi varied here. In case of bhindi seeds the highest number 118 of fungal pathogens was noted which was followed by 82.0 that were found on tomato seeds, 81.0 on spinach, 77.0 on brinjal and lowest on cucumber. This may be correlated with the seed structure of the present vegetable crops. Different fungal pathogens associated with different seeds of vegetables has been reported by different workers such as – Chaudhary *et al*; (2005); Hamim *et al*; (2014); Mancini *et al*; (2016); Patekar *et al*; (2017); and Mahanto *et al*; (2019). Findings of the present works are therefore in agreement with the above findings as except few most of the fungal pathogens were associated with seeds of particular vegetables. The seedlings produced form the different vegetable seeds where fungal association was observed were sown in the laboratory in pre-sterilized sand placed in the plastic tray.

Now % of total seedlings, % of abnormal seedlings, % of diseased seedlings and dead seedlings were calculated on 7^{th}, 14^{th} and 21^{st} day of sowing the mean of the data was placed in table-2,3 and 4. From the table-2, where above parameters were recorded after 7 day of sowing it may be noted that percentage of normal seedlings varied from 46.0 to 76.0. Here the highest percentage of normal seedlings 76.0 was recorded in case of cucumber followed by 74.0 in spinach, egg plant 71.0 in spinach. The lowest percentage, 46.0 of normal seedlings was recorded in bhindi. In case of abnormal seedlings 3.0% was noted in the seedlings of spinach, bhindi and tomato, while 2% in case of seedlings of cucumber and brinjal respectively. In case of diseased seedlings it may be noted that there were no infected seedlings in spinach, while it was 1% in cucumber, 2% in bhindi and egg plant and 3% in tomato only. The maximum dead seedlings 49.0% was noted in case of bhindi followed by 41.0% in tomato and 26.0% in spinach. Minimum dead seedlings were found in case of tomato which was 21.0%.

Germination Test of Different Vegetable Seeds at 14 days after Sowing

Percentage of normal seedlings, abnormal seedlings, diseased seedlings and dead seedlings raised from the different vegetables were also studied after 14^{th} day of showing. The mean of the data was presented in table-3. From the table it may be noted that percentage of normal seedlings varied between 72 to 43%.

Here the percentage of normal seedlings depended on the seed types. Here the highest percentage of normal seedlings was 73.0% in case of cucumber followed by 72.0% in egg plant, 69.0% in spinach. The lowest percentage of normal seedlings 45% was recorded in bhindi. In case of abnormal seedlings highest percentage 4.0% was recorded in bhindi, cucumber and tomato respectively, while it was 3% in case of seedlings of spinach and egg plant. The highest percentage of diseases seedlings was recorded n tomato which was 4.0%. In case of bhindi, cucumber and egg plant it was 3% while in spinach, it was lowest that was 2.0% only.

The maximum dead seedlings 48.0 were noted in case of bhindi while the minimum percentage of dead seedlings was found in case of egg plant that was 21.0% only.

Germination Test of Different Seedlings Raised from Seeds of Different Vegetables after 21 days of Sowing

Mean of data collected for percentage of normal seedlings, abnormal seedlings, diseased seedlings and dead seedlings was placed in table-4. From the table, it may be noted that percentage of normal seedlings varied from 43% to 71%. The highest percentage of normal seedlings was 71% in case of cucumber, followed by egg plant 69% and spinach 67.0% respectively. Here minimum percentage of response 43.0 for the normal seedlings was found in case of bhindi. Percentage of abnormal seedling was also noted. Here 4% of abnormal seedlings were noted in spinach, bhindi, and tomato and egg plant while it was 3% in case of cucumber only. Likewise percentage of diseased seedlings was also recorded. From the table it may be noted that it was 5% in case of cucumber, tomato, and egg plant while 4.0% in bhindi and 3.0% in spinach. The highest percentage 49.0% for the dead seedlings was observed in case of bhindi followed by 42.0% in case of tomato and 26.0% in case of spinach. Lowest percentage of response 21.0% was noted in case of cucumber. Variations in percentage of normal seedlings, abnormal seedlings, diseased seedlings and dead seedling, in different vegetables and other crops have been reported by different workers such as, Puspa *et al*; (1999) in case of different cucurbitaceous plants. Srivastva *et al*; (2011) in case of brassicaceous vegetables. Sharma *et al*; (2013) in case of brinjal, Barry, (2014). Ghosh *et al*; (2018) in cereals crops. Findings of the present work are in agreement with the above findings.

Germination percentage, Mean shoot and root length, vigor index and percentage of germination failure were also recorded. The mean of the data was placed in table-5. From the table, it was noted that percentage of germination ranged between 81.5 to 46.0. Here the maximum percentage of germination 81.5 was found in case of cucumber, followed by 75.6 in case of spinach and 74.5 in case of egg plant. The minimum percentage of response 46.0 was noted in case of bhindi itself. The mean shoot lengths varied between 3.80 cm to 16.50 cm, where the maximum length 16.50 was noted in case of cucumber followed by 12.88 in case of bhindi, 8.35 in case of spinach. Here again minimum length 3.80 was noted in case of egg plant. The mean root length varied between 2.5 to 7.5 cm, where the maximum 7.5 cm was noted in case of cucumber, followed by 7.2 cm in bhindi and 3.21 in spinach. Here also minimum length 2.5 cm was noted in egg plant. The vigor index was calculated as the formula mentioned in the chapter, Materials and Methods. The Vigor Index ranged between 1956 to 469.35. Here the highest vigor index 1956 was noted for cucumber, followed by 923.68 for bhindi, 873.94 for spinach and 530.50 for tomato. The lowest index vigor 469.35 was noted for egg plant. Germination failure of the seeds of above 5 vegetables was also calculated. This was highest 54.0 in case of bhindi, followed by 42.40 in case of tomato, 25.50 in egg plant and 24.4 in spinach. Here lowest percentage was noted in case of cucumber that was 18.5% only.

Table 1: Showing fungal pathogens associated with different seeds of vegetables

	Vegetables on 7th day of sowing				
Fungal species	Spinach	Bhindi	Cucumber	Tomato	Egg Plant
Alternaria spp.	23.0	--	14.0	--	--
Curvularia spp.	15.0	--	17	--	14.0
Fusarium spp.	24.0	22.0	21.0	25.0	20.0
Penicillium spp.	19.0	14.0	10.0	--	--
Colletotrichum spp.	--	21.0	--	--	--
Aspergillus flavus	--	25.0	--	21.0	15.0
Aspergillus niger	--	20	--	15.0	11.0
Phomopsis spp.	--	--	--	--	17.0
Macrophomopsis spp.	--	16	--	--	--
Cladosporium spp.	--	--	--	21	--
Total	81.0	118.0	62.0	82.0	77.0

Table 2: Showing % of normal seedlings, % abnormal seedlings, % of diseased seedlings, % of dead seedlings of different vegetables

Vegetables	% of normal seedlings	% of abnormal seedlings	% of diseased seedlings	% dead seedlings
Spinach	71.0%	3.0%	00%	26.0%
Bhindi	46.0%	3%	2%	49.0%
Cucumber	76.0%	2.0%	1%	21.0%
Tomato	53.0%	3.0%	3%	41.0%
Brinjal egg plant	74.0%	2.0%	2%	22.0%

Table 3: Showing % of normal seedlings, % abnormal seedlings, % of diseased seedlings, %of dead seedlings on 14 day of sowing

Vegetables	% of normal seedlings	% of abnormal seedlings	% of diseased seedlings	% dead seedlings
Spinach	69.0	4.0	2%	26.0
Bhindi	45.0	4.0	3%	48.0
Cucumber	73.0	4.0	3%	20.0
Tomato	51.0	4.0	4.0	41.0
Brinjal egg plant	72.0	3.0	3.0	21.0

Table 4: Showing % of normal seedlings, % abnormal seedlings, % of diseased seedlings, %of dead seedlings on 21 day of sowing

Vegetables	% of normal seedlings	% of abnormal seedlings	% of diseased seedlings	% dead seedlings
Spinach	67.0	4.0	3.0	26.0
Bhindi	43.0	4.0	4.0	49.0
Cucumber	71	4	5.0	21.0
Tomato	49.0	4.0	5.0	42.0
Brinjal egg plant	69.0	4.0	5.0	22.0

Table 5: Showing % germination, mean shoot and root length, vigor index and germination failure of vegetable seeds

Vegetables	% germination	Mean shoot length	Mean root length	Vigor index	Germination failure
Spinach	75.6	8.35	3.21	873.94	24.4
Bhindi	46.0	12.88	7.2	923.68	54.0
Cucumber	81.5	16.50	7.5	1956	18.5
Tomato	57.60	6.35	2.86	530.50	42.40
Brinjal egg plant	74.5	3.80	2.5	469.35	25.50

CONCLUSION

It may be concluded that seed testing before sowing for mycoflora associated with the seeds of vegetables, should be made mandatory before it is marketed. This will reduce the lacks in yield of the respective vegetables.

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