

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(7): 2113-2118
www.biochemjournal.com
Received: 15-05-2026
Accepted: 20-06-2026

PM Chotalia

Department of Plant
Pathology, B. A. College of
Agriculture, Anand
Agricultural University,
Anand, Gujarat, India

VR Gohel

AICRP on National Seed
Project (Crops) – Seed
Technology Research (STR),
Department of Plant
Pathology, B. A. College of
Agriculture, Anand
Agricultural University,
Anand, Gujarat, India

Efficacy of various organic inputs against seed mycoflora of okra cultivar

PM Chotalia and VR Gohel

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7Sy.9369>

Abstract

Okra (*Abelmoschus esculentus* L. Moench) is a widely cultivated vegetable crop valued for its environmental adaptability, high nutritional content, and significant export potential. However, seed-borne pathogens and mycoflora pose serious threats to crop yield and disease management, highlighting the need for eco-friendly precautionary treatments. This study evaluated the efficacy of two organic preparations (*Beejamrut* and cow urine) and two botanical extracts (*Moringa* and neem leaf extracts) against seed-borne mycoflora. Among the tested treatments, *Moringa* leaf extract applied at 10% exhibited the highest colony growth inhibition (44.81%) and restricted fungal diversity to only four species. Additionally, cow urine applied at 3% significantly reduced seed-borne mycoflora incidence to 5.71%, closely followed by 10% *Moringa* leaf extract at 6.74%. These findings demonstrate the potential of *Moringa* leaf extract and cow urine as effective bio-treatments for managing seed-borne fungal infection in okra production.

Keywords: Okra, seed mycoflora, moringa leaf extract, cow urine, bio-efficacy

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is the most commonly grown vegetable crops across the different zones of world. It belongs to family *Malvaceae*. Okra is usually consumed for its green tender fruits as a vegetable in different ways. The mature okra seeds are the good source of oil and protein has been known to have superior nutritional quality. Its mature fruit and stems contain crude fiber, which is used in the paper industry. Okra bast fibre possess an excellent quantity of cellulose, which can be used in cellulose-based industries (Kumar *et al.*, 2013) [7]. In paper industries, the stem of okra plants is used for fibre purposes (Qayyum, 1990; Mithal, 2006). For the clarification of sugarcane juice from which gur or brown sugar is prepared, the roots and stems of okra plants are used (Chauhan, 1972) [5]. Medicinal value of okra has also been reported in curing ulcers and relief from hemorrhoids (Adams, 1975) [2]. Raw fruits and seeds are also rich in mucilage, serves a good source of antioxidants. The seeds of okra when roasted and grounded can be used as coffee additive or as its substitute (Moekchantuk and Kumar, 2004) [9]. Thus, it is indeed an economically important crop.

Fungal pathogens severely threaten okra cultivation worldwide, causing heavy crop losses regardless of regional weather conditions. Seeds of okra affected by various fungi including; *Alternaria alternata*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Stemphylium botryosum*, *Penicillium digitatum*, *Pythium aphanidermatum* (Al-Kassim and Monawar, 2000; Odofin, 2010) [4, 11]. Although chemical fungicides successfully target these pathogens, they leave behind hazardous residues that damage human health and ecosystems. To make matters worse, these fungi are evolving into new, resistant strains that make traditional chemical sprays increasingly useless. This highlights the critical need for alternative control methods. To succeed, these new solutions must be affordable, consistent, biodegradable, and biologically derived. Implementing organic approaches such as priming seeds, applying angiosperm leaf extracts, and using cow-based products offers a practical and eco-friendly way forward. It is an ecological approach using either bacteria or selected fungal antagonists against the soil and seed-borne pathogens (Rai and Basu, 2014) [13]. Nwangburuka *et al.* (2012) [10] studied the effects of moringa leaf extracts on seed germination, seedling growth rate and fungal abundance in okra.

Corresponding Author:**PM Chotalia**

Department of Plant
Pathology, B. A. College of
Agriculture, Anand
Agricultural University,
Anand, Gujarat, India

Abdulhassan (2020) ^[1] evaluated the efficacy of some plant extracts (*Cuminum cymanum*, *Zinziber officinale* and *Citrullus colocynthis*) against seed rotting and seedling death causing fungus *Macrophomina phaseolina* and found that the alcoholic extract of *Cuminum cyminum* had a significant effect on the inhibition of the radial growth and dry weight of *M. phaseolina* followed by *Zingiber officinale* and *Citrullus*. Hence, this research was conducted for evaluating the efficacy of various organic inputs against seed mycoflora of okra cultivar.

Materials and Methods

Experiment details

An *in vitro* experiment conducted for evaluation of various organic inputs viz, *Beejamrut*, cow urine and phytoextracts (moringa and neem leaf extract) for the management of seed mycoflora of okra cultivar i.e., Gujarat Anand Okra-5 (GAO-5) by using agar plate method at Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India.

Methodology

The seeds of okra cultivar (GAO-5) were pre-soaked for 30 minutes with the various organic inputs of different concentrations (3, 5 and 10%) into four repetitions, each of 100 seeds (As per the treatment details of Table 1). Pre-soaked seeds were placed on the agar plates and the growth of various fungi was recorded accordingly.

Preparation of *beejamrut*

Beejamrut was prepared by suspending 5 kg of cow dung (collected from Livestock Research Station, AAU, Anand) in 20 L of water for 12 hours. The cloth was then squeezed to release all soluble ingredients into the suspension, which was subsequently mixed with a separate solution of 50 g of lime in 1 L of water, 5 L of cow urine, and a handful of virgin soil. After incubating for 8–12 hours, the mixture was filtered. The resulting filtrate was diluted with water to formulate 3%, 5%, and 10% concentrations, which were used for a 30-minute pre-soaking seed treatment.

Preparation of phytoextracts

Fresh neem and moringa leaves were washed with distilled water, surface-sterilized with 1% sodium hypochlorite, and homogenized in a mixer grinder. The crude extracts were filtered through a single layer of sterile muslin cloth to serve as 100% stock solutions. These were diluted with water to obtain 3%, 5%, and 10% concentrations, which were then used as a 30-minute pre-soaking seed treatment.

Collection of Cow urine

Cow urine was obtained from the Livestock Research Station, AAU, Anand, and utilized as the 100% standard stock solution. This stock was subsequently diluted with the requisite volume of water to prepare the target treatment concentrations of 3%, 5%, and 10%. The resulting formulations were used to pre-soak the seeds for a duration of 30 minutes.

Table 1: Treatment details for management of seed mycoflora

Treatment No.	Treatments	Dose (per kg seed)
T ₁	<i>Beejamrut</i> @ 3%	500 ml
T ₂	<i>Beejamrut</i> @ 5%	500 ml
T ₃	<i>Beejamrut</i> @ 10%	500 ml
T ₄	Cow urine @ 3%	500 ml
T ₅	Cow urine @ 5%	500 ml
T ₆	Cow urine @ 10%	500 ml
T ₇	Neem leaf extract @ 3%	500 ml
T ₈	Neem leaf extract @ 5%	500 ml
T ₉	Neem leaf extract @ 10%	500 ml
T ₁₀	Moringa leaf extract @ 3%	500 ml
T ₁₁	Moringa leaf extract @ 5%	500 ml
T ₁₂	Moringa leaf extract @ 10%	500 ml
T ₁₃	Untreated check (Control)	-

Bioassay of the Dominant Seed Mycoflora against Various Organic Inputs

Experiment details

An *in vitro* bioassay was conducted using the poisoned food technique to evaluate the treatments (As per the treatment details of Table 1) against the dominant mycoflora isolated from the okra cultivars at Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India.

Observation of the radial growth was recorded from 24 hrs. of the incubation to the complete growth of test pathogens in control plates obtained. The per cent growth inhibition over control was calculated using formula given by Vincent (1947) ^[15].

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where,

PGI= Per cent growth inhibition

DC = Mean diameter of mycelial colony in control treatment (mm)

DT = Mean diameter of mycelial colony in treated set (mm)

Methodology

In Vitro Evaluation via Poisoned Food Technique

To evaluate the organic inputs *in vitro*, specific quantities of each treatment were individually incorporated into conical flasks containing melted, sterile Potato Dextrose Agar (PDA) to achieve the target concentrations (e.g., 3 mL of input in 97 mL of PDA for a 3% concentration). The poisoned media were thoroughly agitated to ensure uniform distribution before 20 mL portions were poured into sterile Petri plates. Control plates were prepared simultaneously using unamended PDA medium. Upon solidification, each plate was centrally inoculated with a 5 mm diameter culture disc, cut aseptically using a cork-borer from a 7-10 days old

pure culture of the dominant seed mycoflora isolated from okra cultivars. Each treatment was maintained in triplicate. The inoculated plates were incubated in a BOD incubator at 25 + 2 °C until the test pathogen reached full growth in the control plates, with colony diameters recorded periodically.

Results

Significant variation was recorded in the percentage of seeds infected by seed-borne mycoflora across different organic inputs (Table 2). Application of cow urine @ 3% (T₄) proved most effective in suppressing overall mycoflora incidence, recording the minimum seed infestation of 5.72%, which was closely followed by cow urine treatments at 5% (7.10%) and 10% (7.28%). In contrast, the highest seed mycoflora prevalence (12.24%) was observed in seeds treated with *Beejamrut* @ 3% (T₁). Qualitative evaluation of seeds treated with cow urine @ 10% (T₆) revealed the presence of only four predominant fungal taxa: *Aspergillus flavus* (2.43%), *A. fischeri* (2.08%), *Fusarium oxysporum* f. sp. *vasinfectum* (1.45%), and *A. niger* (1.33%). Seed

treatment with cow urine @ 10% (T₆) completely suppressed the growth of *Macrophomina phaseolina*, *Colletotrichum dematium*, *Aspergillus terreus*, and *A. tamarii*. Similarly, cow urine @ 3% (T₄) achieved 100% inhibition of *C. dematium*, *A. terreus*, and *A. tamarii*. Furthermore, *Beejamrut* @ 10% (T₃) exhibited complete efficacy (100% inhibition) against *Aspergillus fischeri* and *A. tamarii*. (Plate 1)

Significant variation was observed in the antifungal efficacy of the evaluated phytoextracts (Plate 2). Moringa leaf extract @ 10% (T₁₂) recorded the lowest seed infection rate (6.74%) and the lowest species richness (4 species), followed by Moringa leaf extract @ 5% (7.03%), Neem leaf extract @ 10% (9.43%), and Moringa leaf extract @ 3% (9.93%).

Complete inhibition (100%) of *C. dematium*, *A. flavus*, *A. terreus*, and *A. tamarii* was observed in seeds treated with Moringa leaf extract @ 10% (T₁₂). Similarly, treatment with Neem leaf extract @ 10% (T₉) completely eliminated the growth of *M. phaseolina*, *A. niger*, and *A. terreus*.

Table 2: *In vitro* management of seed mycoflora of okra (GAO 5)

Tr. no.	Treatments	Dose (per kg seeds)	Seeds showing mycoflora (%)								Total fungal species observed	Total (%)
			<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	<i>Macrophomina phaseolina</i>	<i>Colletotrichum dematium</i>	<i>Aspergillus fischeri</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus tamarii</i>		
T ₁	<i>Beejamrut</i> @ 3%	500 ml	1.24	3.31	2.15	1.40	1.45	1.49	0.24	0.96	8	12.24
T ₂	<i>Beejamrut</i> @ 5%	500 ml	4.30	2.21	0.00	1.19	1.15	0.88	1.23	0.80	7	11.75
T ₃	<i>Beejamrut</i> @ 10%	500 ml	2.25	0.94	1.38	0.00	2.89	1.18	0.96	0.00	6	9.59
T ₄	Cow urine @ 3%	500 ml	1.14	0.65	0.00	1.91	1.18	0.84	0.00	0.00	5	5.72
T ₅	Cow urine @ 5%	500 ml	2.21	1.39	1.34	0.46	0.25	0.79	0.15	0.51	8	7.10
T ₆	Cow urine @ 10%	500 ml	1.45	0.00	0.00	2.08	1.33	2.43	0.00	0.00	4	7.28
T ₇	Neem leaf extract @ 3%	500 ml	1.23	3.19	2.08	1.71	2.79	2.05	0.27	0.96	8	14.27
T ₈	Neem leaf extract @ 5%	500 ml	2.80	2.15	1.74	0.00	0.43	3.75	0.15	0.85	7	11.86
T ₉	Neem leaf extract @ 10%	500 ml	2.38	0.00	2.14	2.64	0.00	1.93	0.00	0.35	5	9.43
T ₁₀	Moringa leaf extract @ 3%	500 ml	3.73	1.99	0.39	0.63	1.56	0.15	1.32	0.18	8	9.93
T ₁₁	Moringa leaf extract @ 5%	500 ml	2.66	0.80	0.41	0.81	0.38	1.28	0.70	0.00	7	7.04
T ₁₂	Moringa leaf extract @ 10%	500 ml	3.33	0.93	0.00	1.35	1.14	0.00	0.00	0.00	4	6.74
T ₁₃	Untreated (Control)	-	5.87	3.90	3.10	2.39	4.46	2.78	1.48	2.10	8	26.07
S. Em. ± C. D. at 5 % C. V. (%)			0.10	0.06	0.05	0.05	0.06	0.06	0.02	0.02		
			0.30	0.18	0.13	0.13	0.16	0.17	0.06	0.07		
			7.80	7.70	8.00	7.07	7.51	8.05	8.44	8.94		

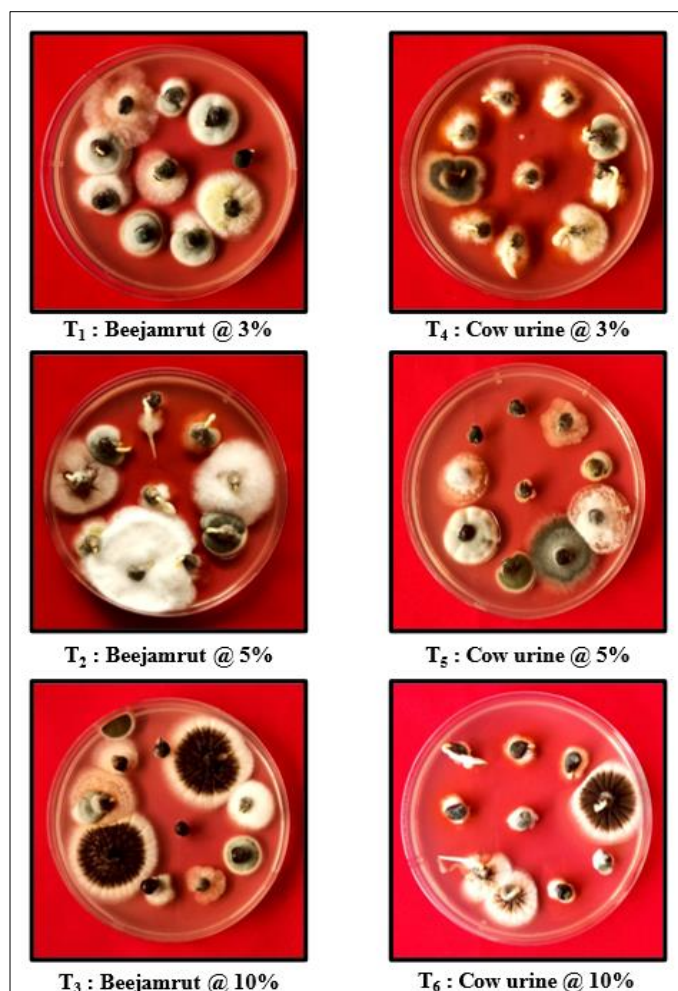


Plate 1: *In vitro* management of seed mycoflora

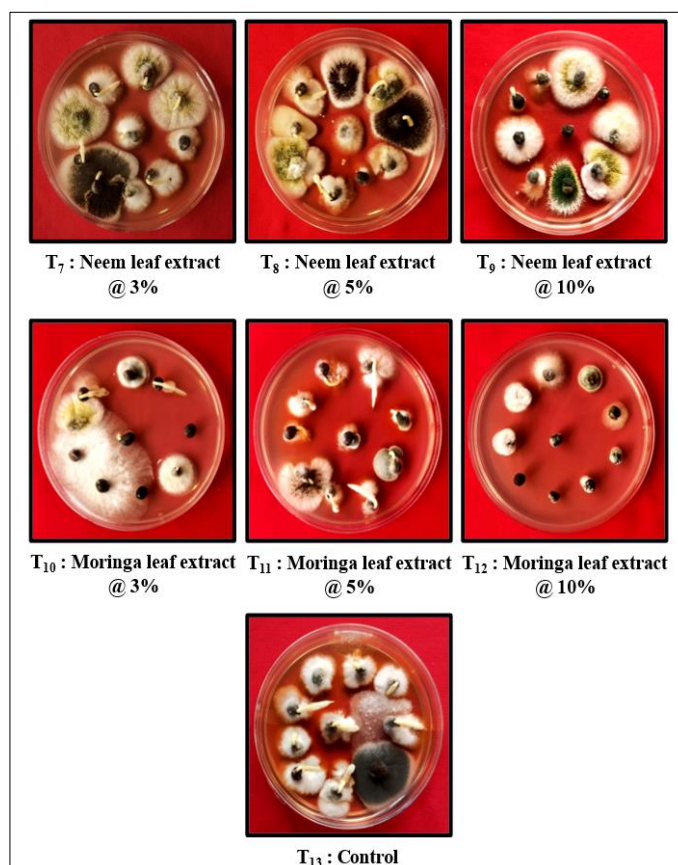


Plate 2: *In vitro* management of seed mycoflora

Bioassay of the Dominant Seed Mycoflora against Various Organic Inputs

In vitro evaluation of organic inputs

To assess the antifungal potential of various organic inputs against the principal seed mycoflora of okra (*Fusarium oxysporum* f. sp. *vasinfectum*), an *in vitro* assay was conducted using the poisoned food technique. The treatments exhibited significant variation in suppressing mycelial growth.

Evaluation of treatments against the test pathogen demonstrated significant variation in mycelial growth inhibition (Table 3). Among all evaluated treatments, Moringa leaf extract @ 10% (T₁₂) exhibited superior antifungal activity, achieving the highest growth inhibition at 44.81%. This was followed by Neem leaf extract @ 10% (T₉) (37.41%) and Moringa leaf extract @ 5% (T₁₁) (36.67%). (Plate 3)

Table 3: Efficacy of various organic inputs against dominant seed mycoflora (*Fusarium oxysporum* f. sp. *vasinfectum*) of okra under *in vitro* condition

Tr. No.	Treatments	Mycelial growth (mm)	Growth inhibition (%)
T ₁	Beejamrut @ 3%	70.33	21.85
T ₂	Beejamrut @ 5%	66.33	26.30
T ₃	Beejamrut @ 10%	68.00	24.44
T ₄	Cow urine @ 3%	73.33	18.52
T ₅	Cow urine @ 5%	70.00	22.22
T ₆	Cow urine @ 10%	69.33	22.96
T ₇	Neem leaf extract @ 3%	65.33	27.41
T ₈	Neem leaf extract @ 5%	62.00	31.11
T ₉	Neem leaf extract @ 10%	56.33	37.41
T ₁₀	Moringa leaf extract @ 3%	59.67	33.70
T ₁₁	Moringa leaf extract @ 5%	57.00	36.67
T ₁₂	Moringa leaf extract @ 10%	49.67	44.81
T ₁₃	Untreated (Control)	90.00	-
S. Em. ±		1.11	
C. D. at 5 %		3.21	
C. V. (%)		2.90	

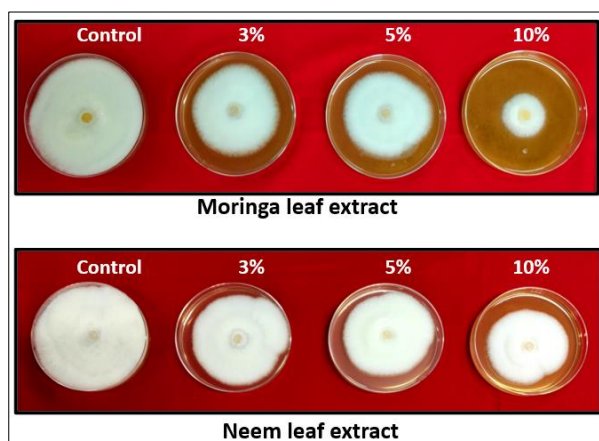


Plate 3: Efficacy of organic inputs against dominant seed mycoflora (*Fusarium oxysporum* f. sp. *vasinfectum*)

Discussion

The present study highlights the significant biological potential of traditional organic inputs and botanical extracts in suppressing seed-borne mycoflora and controlling major phytopathogenic fungi in okra. Evaluation of organic treatments (*Beejamrut* and cow urine) alongside botanical extracts (*Moringa oleifera* and *Azadirachta indica*) across varying concentrations (3%, 5%, and 10%) demonstrated concentration-dependent antifungal efficacy under *in vitro* conditions.

Among the organic inputs, cow urine proved highly effective in restricting both total mycoflora incidence and fungal species richness. Treatment with cow urine @ 3% recorded the lowest overall seed infestation (5.71%), while cow urine @ 10% restricted species diversity to just four persistent fungal taxa: *Fusarium oxysporum* f. sp. *vasinfectum* (1.45%), *Aspergillus fischeri* (2.08%), *A. niger* (1.33%), and *A. flavus* (2.43%). The broad-spectrum

fungicidal action of cow urine; evidenced by the complete inhibition of *Macrophomina phaseolina*, *Colletotrichum dematium*, *A. terreus*, and *A. tamarii* can be attributed to its bio-active constituents. Cow urine naturally contains phenols, uric acid, hippuric acid, nitrogen, and essential salts, which create an unfavorable micro-environment for fungal spore germination and mycelial proliferation (Randhawa, 2010)^[14].

Regarding botanical inputs, Moringa leaf extract @ 10% demonstrated superior antifungal performance compared to neem extract, exhibiting the lowest species richness (4 species) and minimum total seed mycoflora incidence (6.74%). Furthermore, Moringa leaf extract @ 10% completely suppressed (100% inhibition) key storage and field pathogens, including *C. dematium*, *A. flavus*, *A. terreus*, and *A. tamarii*. Neem leaf extract @ 10% also showed potent selective efficacy, achieving complete eradication of *M. phaseolina*, *A. niger*, and *A. terreus*. The

strong antifungal properties of *Moringa oleifera* are primarily linked to its rich profile of secondary metabolites, particularly glucosinolates, isothiocyanates, and polyphenolic compounds, which disrupt fungal cellular membranes and inhibit enzymatic pathways essential for hyphal expansion (Adewole, 2024; Kubheka *et al.*, 2020) [3, 6].

When evaluated against the dominant seed-borne pathogen of okra, *F. oxysporum* f. sp. *vasinfectum*, using the poisoned food technique, *Moringa* leaf extract @ 10% outperformed all other treatments, recording the highest mycelial growth inhibition (44.81%). These findings underscore the potential of *Moringa* leaf extract as an eco-friendly bio-fungicide and seed-dressing agent, offering a sustainable, non-chemical alternative to synthetic fungicides for managing seed-borne vascular wilt pathogens in okra cultivation.

Conclusion

In conclusion, the present study demonstrates the significant potential of organic inputs and botanical extracts as sustainable, eco-friendly alternatives to chemical fungicides for managing seed mycoflora in okra.

References

1. Abdulhassan HA. The efficiency of some plant extracts on the fungus *Macrophomina phaseolina*, the causal agent of seed rot and okra seedling death. IOP Conference Series: Earth and Environmental Science. 2020;553(1):012046.
2. Adams CF. Nutritive value of American foods in common units. Agriculture Handbook No. 425. Washington (DC): U.S. Department of Agriculture; 1975. p. 29.
3. Adewole O. Plant-based antifungal agents: Prospects, exploitation and utilization. In: Phytochemicals and Plant Pathology. London: IntechOpen; 2024.
4. Al-Kassim MY, Monawar MN. Seed-borne fungi of some vegetable seeds in Gazan Province and their chemical control. Saudi Journal of Biological Sciences. 2000;7(2):179-184.
5. Chauhan DVS. Vegetable Production in India. 3rd ed. Agra: Ram Prasad and Sons; 1972.
6. Kubheka SF, Tesfay SZ, Mditshwa A, Magwaza LS. Evaluating the efficacy of edible coatings incorporated with *Moringa* leaf extract on postharvest quality of 'Maluma' avocado fruit and its biofungicidal effect. HortScience. 2020;55(4):410-415.
7. Kumar DS, Tony DE, Kumar AP, Kumar KA, Rao DB, Nadendla R. A review on *Abelmoschus esculentus* (okra). International Research Journal of Pharmacy and Applied Sciences. 2013;3(4):129-132.
8. Mithal MJ. Low cost and pollution free technology against root rot of okra. Pakistan Journal of Plant Pathology. 2006;1(6):1-6.
9. Moekchantuk T, Kumar P. Export okra production in Thailand. Bangkok: Food and Agriculture Organization of the United Nations; 2004. Inter-country Programme for Vegetable IPM in South and Southeast Asia Phase II. 56 p.
10. Nwangburuka CC, Oyekale K, Ezekiel CN, Anokwuru PC, Badaru O. Effects of *Moringa oleifera* leaf extract and sodium hypochlorite seed pre-treatment on seed germination, seedling growth rate and fungal abundance in two accessions of *Abelmoschus esculentus* (L.) Moench. Archives of Applied Science Research. 2012;4(2):875-881.
11. Odofin O. The effect of neem extract and sodium hypochlorite pre-treatment on germination, fungal growth and mitotic index of *Abelmoschus esculentus* L. (okra) [doctoral dissertation]. Ilishan-Remo (Nigeria): Babcock University; 2010.
12. Qayyum S. A varietal trial on okra (*Hibiscus esculentus* L.) cultivars. Pakistan Journal of Agriculture. 1990;7(4):55-78.
13. Rai AK, Basu AK. Pre-sowing seed bio-priming in okra: Response for seed production. Bioscan. 2014;9(2):643-647.
14. Randhawa GK. Cow urine distillate as bioenhancer. Journal of Ayurveda and Integrative Medicine. 2010;1(4):240-241.
15. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159(4051):850-850.