The Pharma Innovation Journal 2023; 12(7): 3750-3754

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 3750-3754

www.thepharmajournal.com
Received: 20-05-2023
Accepted: 26-06-2023

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Screening of chemical fungicides against *Fusarium incarnatum-equiseti* inciting pod rot of mungbean [*Vigna radiata* (L.) Wilczek] under *in vitro* condition

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Abstract
Pod rot of mungbean caused by *Fusarium incarnatum-equiseti* is a newly emerged fungal disease of mungbean that caused bottleneck reduction in production. In the present study nine different fungicides *viz*. Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin, Azoxystrobin + Difenoconazole, Carboxin + Thiram, Metiram + Pyraclostrobin, Hexaconazole, Chlorothalonil, Propiconazole, and Tebuconazole were evaluated under *in vitro* conditions for their antifungal potential against *Fusarium incarnatum-equiseti.* The fungicides were tested at different concentrations *i.e.*, 15, 25, 50, 100, and 150 ppm concentrations employing the "Poisoned Food Technique". Among the fungicides, Tebuconazole and Propiconazole resulted in maximum mycelial growth inhibition with 80.23% and 74.13% at 150 ppm concentration, respectively. While the lowest percent inhibition was recorded in Hexaconazole 65.16 percent, over the control. Among the different combi fungicides, 100% inhibition of mycelial growth was recorded in Azoxystrobin + difenoconazole and Tebuconazole + Trifloxystrobin at 150 ppm followed by Carboxin + Thiram and Metiram + pyraclostrobin with 61.18% and 52.22%, respectively, and the least percent inhibition was observed in Carbendazim + mancozeb with 39.40% at 150 ppm concentration.

Keywords: Pod rot, *Fusarium incarnatum-equiseti*, mungbean, fungicide

Introduction
Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most significant pulse crops, belongs to the family Leguminosae, and is commonly known as green gram it is also known as "Golden gram" because of its nutritional richness. According to Vavilov (1926) [20], mungbean is thought to have originated from the Indian subcontinent and domesticated as early as 1500 BC. Cultivated mungbean was introduced to southern and eastern Asia, Africa, Austronesia, the Americas, and the West Indies. It is now widespread throughout the Tropics and is found from sea level up to an altitude of 1850 m in the Himalayas.

Mungbean is an economically significant grain legume crop that is consumed by humans as food and by an animal as feed. There are 3 subgroups of *Vigna radiata*: one is cultivated (*Vigna radiata* sub sp. *radiata*), and two are wild (*Vigna radiata* sub sp. *sublobata* and *Vigna radiata* subsp. *glabra*). In India mungbean is grown in almost all the states due to its triple use i.e., food, fodder, and for improving soil fertility. mungbean seeds have a protein content of about 20-24% and thus act as a good source of protein (Keatinge *et al*., 2011) [17] these proteins are high in essential amino acids such as leucine, lysine and phenylalanine/tyrosine, and valine, isoleucine, and histidine that are lacking in cereals grains. mungbean also contains carbohydrates (56%), dietary fibre (16.3%), fat (1.3%), phosphorus (124 mg/100 g), calcium (326 mg/100 g), minerals (3.5%), Iron (7.3 mg/100 g) and moisture of about 10% crops.

Mungbean is grown primarily in tropical and subtropical areas such as India, China, Bangladesh, Myanmar, Indonesia, Thailand, and some parts of central and eastern Africa, the United States of America, the West Indies, and Australia (Westphal, 1974). mungbean is cultivated on more than 6 million hectares of land worldwide (about 8.5% of global pulse cultivation area) and global annual production is about 3 million tons (5% of global pulse production). Globally, India is the largest producer and consumer of mungbean (Nair *et al*., 2014) [17] holding about 35% area and production of about 25% worldwide with 2.17 MT production followed by China with 0.98 million tonnes and Myanmar with 0.400 MT production (DAC and FW, 2017). Mungbean production in India has increased in recent years with an estimate of about 40.43 lakh hectares, producing 19.48 lakh tonnes of grains with a

productivity of 483 kg per hectare (DAC and FW, 2021). The largest producing state are Rajasthan (16.16 lakh ha), Maharashtra (4.08 lakh ha), Madhya Pradesh (3.54 lakh ha), Karnataka (3.70 lakh ha), Tamil Nadu (1.97 lakh ha), Bihar (1.71 lakh ha), and Andhra Pradesh (1.55 lakh ha), which is about 80% of the total area. Among the mungbean-grown states, the highest production of about 7.66 lakh tonnes in Rajasthan followed by Madhya Pradesh (2.19 lakh tonnes) and Maharashtra (1.55 lakh tonnes), Bihar (1.22 lakh tonnes) and the state with the highest productivity is Andhra Pradesh (670 kg/ha) followed by Bihar (652 kg/ha) and Madhya Pradesh (621kg/ha) (DAC and FW, 2021). In recent years pod rot has emerged as a major bottleneck disease in mungbean production. The disease symptoms appear as discoloration of seeds with rotting of pods and seeds, symptoms become more severe with the increase in relative humidity and rainfall at the time of maturity. In past studies, there is no prior information available on the pod rot of mungbean but in the case of other crops, many microorganisms such as *Rhizoctonia*, *Pythium spp*., *Fusarium spp*., *Aspergillus spp*., etc. are responsible for rotting of pods. Many effective management practices have been used to manage pod rot as pods are the most economical part of the crop (Chaudhary *et al.*, 2011) [1]. Many fungicide applications are being extensively used to manage the disease such as the use of seed treatment, and foliar application of fungicide but these are hazardous, uneconomical, disturb the ecological balance, have a residual effect on food crops, and result in the development of resistance. The cultural and physical methods to manage disease were found effective in reducing the disease. Despite, all the efforts and various management measures for the disease, no effective economic control has been developed so far. This could be due to a lack of proper knowledge about the etiology of the pathogen and the involvement of several species of microorganisms that have been reported to cause the infection alone or together so it is difficult to identify the primary pathogen of the disease. Hence present study investigates the *in-vitro* efficacy of different fungicides against *Fusarium incarnatum-equiseti* Causing pod rot of mungbean.

Materials and Methods

Collection of disease samples

The progression of symptoms on naturally infected pods was carefully observed from disease onset to crop harvest at regular intervals under field conditions at the N.E. Borlaug Crops Research Center (CRC), GBPUAT, Pantnagar. The diseased samples were brought to the laboratory for isolation of the pathogen.

Isolation of pathogen

For isolation of the pathogen, infected pods were collected from the pulse pathology block of N.E. Borlaug Crop Research Center, GBPUA and T. The infected pods were first washed with tap water to remove dirt and soil particles. The samples were then dried by placing them on blotter paper. After drying, pods were cut into 1-2 mm small pieces, and surface sterilization was done by dipping the pod piece in a 2 percent sodium hypochlorite solution, followed by washing it 2-3 times in distilled water, and drying it on sterilized blotter paper. The well-dried samples were placed on Petri plates containing Potato dextrose media under aseptic conditions, and these were kept in a B.O.D (Biological Oxygen Demand) incubator at a temperature of 28±1 °C for the growth of the pathogen.

Evaluation of fungicides

The poison food technique given by Nene and Thapliyal (1993) [3] was used to investigate the efficacy of the fungi toxicants in laboratory conditions. All the fungicides (Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin, Azoxystrobin + Difenoconazole, Carboxin + Thiram, Metiram + Pyraclostrobin, Hexaconazole, Propiconazole, Tebuconazole, Chlorothalonil) were tested at different concentration *viz.* 15, 25, 50, 100, and 150 ppm. The required amount of the test chemical was mixed with 100 ml of sterilized CMA media, and the poisoned medium was poured onto Petri plates (90 mm diameter) under aseptic conditions. Circular bits (5 mm) cut from a 7-day-old culture using sterilized sharp cork borer of the actively growing fungus were inoculated aseptically in the center of each Petri plate, and each concentration was repeated three times. Petri dishes with CMA medium without fungicide were used as controls. After inoculation, the plates were kept in B.O.D at 30±1 °C. The radial growth of the pathogen was recorded when the growth in the control plate was full (*i.e.*, 90 mm) and percent inhibition in colony growth (Pi) was calculated using the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,
C = Radial growth in control (mm)
T = Radial growth diameter in treatment
I = Percent Inhibition

Statistical analysis: All the data were analyzed statistically by using OPSTAT software was compared by mean of critical difference at a 0.05% level of significance.

Results and Discussion

Nine different fungicides *viz***.** Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin, Azoxystrobin + Difenoconazole, Carboxin + Thiram, Metiram + Pyraclostrobin, Hexaconazole, Chlorothalonil, Propiconazole, and Tebuconazole were evaluated under *in vitro* conditions to assess the antifungal activity against *Fusarium incarnatum-equiseti.* The fungicides were tested at different concentrations *i.e.*, 15, 25, 50, 100, and 150 ppm concentrations using the "Poisoned Food Technique" on the CMA medium. All the fungicides significantly differ as compared to the control as well as with one another in reducing the radial growth of the fungus except Propiconazole and Tebuconazole at 10 ppm, the increase in the percentage of inhibition was directly proportional to the increase in the concentration of fungicides.

At 15 ppm concentration maximum growth inhibition of 65.59 percent was recorded in Tebuconazole + Trifloxystrobin followed by propiconazole (63.80%), Tebuconazole (52.78%), Azoxystrobin + difenoconazole (45.26%), and Metiram + pyraclostrobin (31.48%), While least mycelium growth inhibition was observed in Chlorothalonil (23.44%) followed by Carbendazim + mancozeb (28.60%), Hexaconazole (30.28%), and Carboxin + Thiram (30.74%), respectively after 8 day of incubation over the control.

At 25 ppm concentration, maximum growth inhibition was recorded in Tebuconazole + Trifloxystrobin (70.27%) followed by Azoxystrobin + difenoconazole (66.28%), Propiconazole (65.33) Tebuconazole (65.26%), and Carbendazim + mancozeb (38.52%), respectively. While the least mycelium growth inhibition was recorded in Chlorothalonil (26.45%) followed by Carboxin + Thiram (37.02%), Metiram + pyraclostrobin (38.52%), and Hexaconazole (40.39%), respectively over the control.

At 50 ppm concentration, maximum growth inhibition was recorded in Tebuconazole + Trifloxystrobin (84.55%) followed by, Azoxystrobin + difenoconazole (76.92%), Tebuconazole (70.52%), Propiconazole (66.21%), and Hexaconazole (60.46%). The lowest percent inhibition was observed in Chlorothalonil (32.37%) followed by Metiram + pyraclostrobin (41.48%), Carboxin + Thiram (48.64%), Carbendazim + mancozeb (51.09%), respectively over the control.

At 100 ppm concentration, maximum growth inhibition was observed in Tebuconazole + Trifloxystrobin (100%) followed by Azoxystrobin + difenoconazole (100%), Tebuconazole (73.31%), Propiconazole (67.47%), and Hexaconazole (63.17%). The least mycelium growth inhibition was observed in Chlorothalonil (36.37%) followed by Metiram + pyraclostrobin (45.56%), Carboxin + Thiram (56.18%), and Carbendazim + Mancozeb (57.48%) respectively over the control.

At 150 ppm concentration, maximum inhibition was recorded in Tebuconazole + Trifloxystrobin with cent percent (100%) followed by Azoxystrobin + difenoconazole (100%), Tebuconazole (80.23%), Propiconazole (74.13%), and Hexaconazole (65.16%). The least mycelium growth inhibition was recorded in Chlorothalonil (39.40%) followed by Metiram + pyraclostrobin (52.22%), Carboxin + Thiram (61.18%), and Carbendazim + Mancozeb (63.68%), respectively. Similar results were observed by Vineeth *et al.*, (2022) [7] who reported Chlorothalonil showed the least mycelium growth inhibition. Poussio *et al.*, (2021) also reported Nativo (Tebuconazole + Trifloxystrobin) showed the highest mycelium growth inhibition of the *F. oxysporum* f.sp. *lycopersici i.e.*, 95.55%, and 88.88% at 1000 and 500 ppm, respectively. Niwas *et al*. (2020) [13] reported carbendazim, at 500 and 750 ppm completely inhibited the growth of *F. oxysporum* f. sp. *cubense* followed by Azoxystrobin *i.e.*, 32.96, 11.30, 8.12, and 7.16 mm growth observed in 100, 250, 500 and 750 ppm, respectively.

Table 1: Evaluation of different fungicides on radial growth and percent inhibition of *Fusarium incarnatum-equiseti* at 30 ±1 °C

S. No	Treatments	Concentrations (ppm)									
		*Mycelial growth (mm)					*Inhibition over control (%)				
		15	25	50	100	150	15	25	50	100	150
1	Carboxin + Thiram	62.34	56.69	46.22	39.44	34.94	30.74	37.02	48.64	56.18	61.18
2	Propiconazole	32.58	31.20	30.41	29.28	23.28	63.80	65.33	66.21	67.47	74.13
3	Tebuconazole	42.50	31.27	26.53	24.02	17.80	52.78	65.26	70.52	73.31	80.23
4	Chlorothalonil	68.91	66.20	60.87	57.27	54.54	23.44	26.45	32.37	36.37	39.40
5	Hexaconazole	62.75	53.65	35.59	33.15	31.36	30.28	40.39	60.46	63.17	65.16
6	Azoxystrobin + Difenoconazole	49.27	30.35	20.77	0.00	0.00	45.26	66.28	76.92	100.00	100.00
7	Carbendazim + Mancozeb	64.26	53.57	44.02	38.27	32.69	28.60	40.48	51.09	57.48	63.68
8	Metiram + Pyraclostrobin	61.67	55.33	52.67	49.00	43.00	31.48	38.52	41.48	45.56	52.22
9	Tebuconazole + Trifloxystrobin	30.97	26.76	13.90	0.00	0.00	65.59	70.27	84.55	100.00	100.00
10	Control	90	90	90	90	90	0	0	0	0	0
		Treatments (a)			Concentrations (b)		Interactions (a x b)				
	S.Em.±	0.30			0.21		1.92				
	C.D.(P=0.05)	0.86			0.61		0.68				

*Mean of three replications

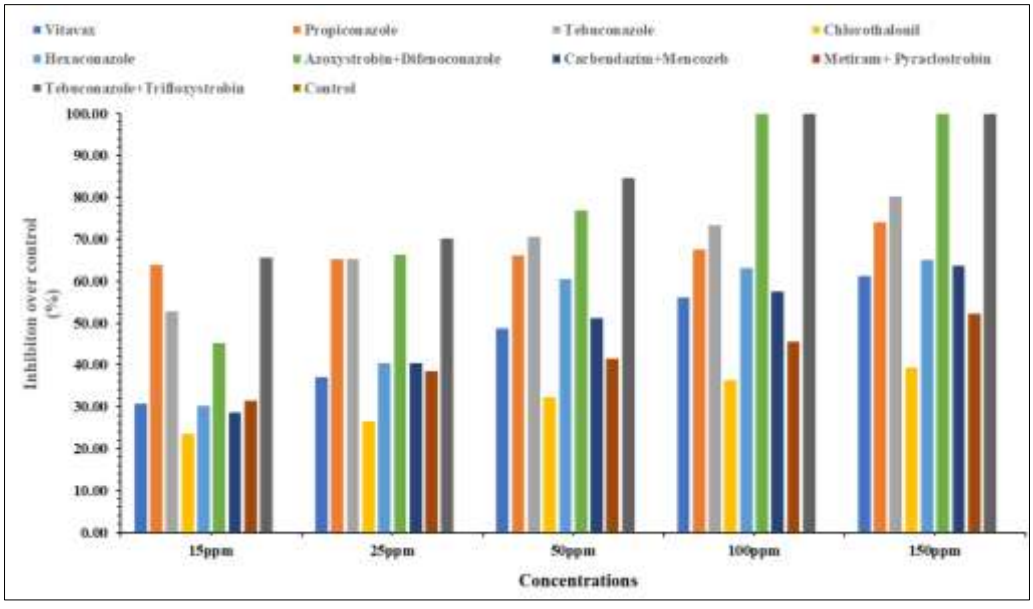

Fig 1: Efficacy of different fungicides on radial growth inhibition of *Fusarium incarnatum-equiseti* at 30±1 °C

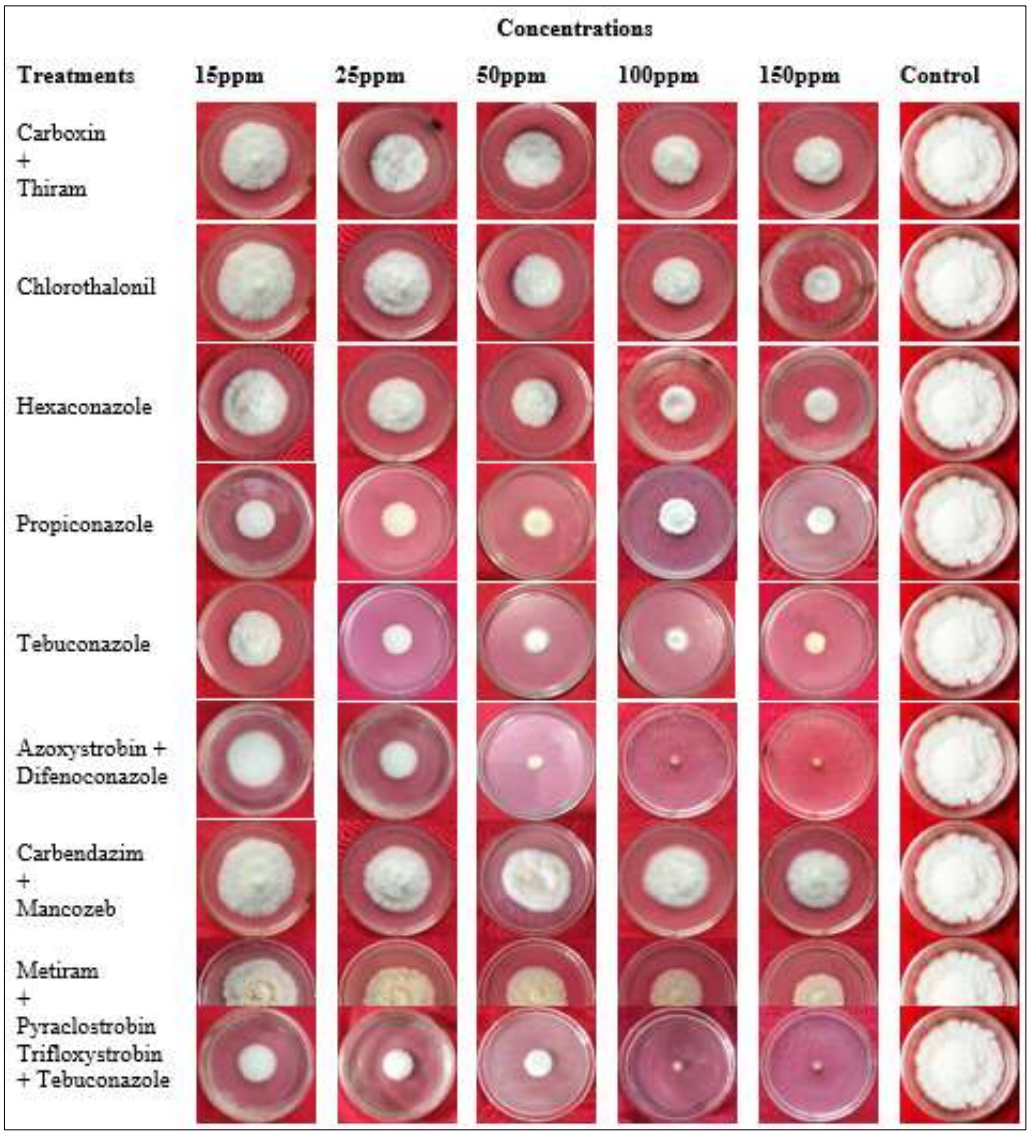

Plate 1: Effect of fungicides on radial growth of *Fusarium incarnatum-equiseti* at 30±1 °C

Conclusion

Among the solo fungicides, Tebuconazole and Propiconazole have resulted in maximum mycelial growth inhibition with 80.23% and 74.13% at 150 ppm concentration respectively, and the least percent inhibition was observed in Hexaconazole 65.16%. Among the different combi fungicides cent percent mycelial growth inhibition was found in Azoxystrobin + difenoconazole and Tebuconazole + Trifloxystrobin at 150 ppm concentration, followed by Carboxin + Thiram and Metiram + pyraclostrobin with 61.18% and 52.22% respectively, and the least percent inhibition of mycelial growth was observed in Carbendazim + mancozeb with 39.40% at 150 ppm concentration.

References

1. Chaudhary B, Kumar S, Kushwaha SK. Evaluation of plant extracts and fungicides against *Fusarium udum* causing pigeonpea wilt. Chem Sci Rev Lett. 2019;8(32):340-344.
2. Dahal N, Shrestha RK. Evaluation of efficacy of fungicides against *Fusarium oxysporum* f. sp. *lentis in vitro* at Lamjung, Nepal. Journal of the Institute of Agriculture and Animal Science. 2018;35(1):105-112.
3. Nene YL, Thapliyal PN. Fungicides in plant disease control (No. Ed. 3). International Science Publisher, 1993.
4. Patel M, Kumar S, Mishra S. Comparative efficacy of combi fungicides and solo fungicides against *Fusarium udum* causing wilt of pigeonpea. Pharma Innovation. 2021;10(5):1310-1314.
5. Singh S, Sinha A, Mishra J. Evaluation of different treatment on the occurrences of seed borne fungi of mungbean *Vigna radiata* (L.) Wilczek seed. Afr. J Agric. Res. 2014;9(44):3300-3304.
6. Vani MS, Kumar S, Gulya R. *In vitro* evaluation of fungicides and plant extracts against *Fusarium oxysporum* causing wilt of mungbean. Pharma Innov. 2019;8:297-302.
7. Vineeth M, Ekabote SD, Naik GR, Pruthviraj RA, Kerure P. *In vitro* evaluation of fungicides against *Fusarium equiseti* causing blight of tuberose. Pharma. Innova. 2022;11(3):1170-1173.
8. Gupta RP, Singh SK, Singh RV. Assessment of losses due to web blight and weather effects on disease development in mungbean. Indian Phytopathology. 2010;63(1):108.
9. Shah MI, Phalisteen S, Nasier A, Williams P, Arif J, Sajad M, *et al*. *In vitro* study on effect of some fungicides

viz. Carbendazim, Mancozeb, conjoint Carbendazim Mancozeb and Sulphur against *F*. *oxysporum*. Research Journal of Microbiology. 2010;5(10):1052-1057.
10. Yadav LB, Kushwaha K. Efficacy of Seed Dressing Agents and Foliar Spray of Fungicides Against Web Blight of Mungbean [*Vigna radiata* (L.) Hepper]. Advances. 2016, 5474.
11. Selvi KV, Sivakumar T. Original Research Article Isolation, identification and Characterization of *Fusarium* species from mangrove habitat of Pichavaram, Tamil Nadu. India International Journal of Current Microbiology and Applied Sciences. 2013;2:33-49.
12. Senapati AK, Ghose S. Screening of ginger varieties against rhizome rot disease complex in eastern ghat high land zone of Orissa. Indian Phytopathology. 2005;58(4):437.
13. Niwas R, Chand G, Azad C. *In vitro* evaluation of fungicides against growth of *Fusarium oxysporum* f. sp. *cubense* causing panama wilt disease of Banana. Int J Chem Stud. 2020;8:130-3.
14. Poussio GB, Abro MA, Syed RN, Khaskheli MI, Jiskani AM. *In vitro* Chemical Management of *Fusarium* Wilt of Tomato in Sindh, Pakistan. vitro. 2021;27:28.
15. India. Ministry of Agriculture and Farmers Welfare, Department of Agriculture, Cooperation and Farmer welfare. Pulses in India, Retrospect and Prospects, Bhopal, 2017, 50.
16. Keatinge JDH, Easdown WJ, Yang RY, Chadha ML, Shanmugasundaram S. Overcoming chronic malnutrition in a future warming world: the key importance of mungbean and vegetable soybean. Euphytica. 2011;180:129-141.
17. Gopalakrishnan Nair PM, Kim SH, Chung IM. Copper oxide nanoparticle toxicity in mungbean (*Vigna radiata* L.) seedlings: physiological and molecular level responses of *in vitro* grown plants. Acta Physiologiae Plantarum. 2014;36:2947-2958.
18. Choudhary HR, Sharma OP, Yadav LR, Choudhary GL. Effect of organic sources and chemical fertilizers on productivity of mungbean. Journal of Food Legumes. 2011;24(4):324-326.
19. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159(4051):850-850.
20. Vavilov NI. Studies on the Origin of Cultivated Plants Institut de Botanique Appliquée et d'Amélioration des Plantes; c1926.