Agricultural Sciences Journal
Available online at http://asj.mnsuam.edu.pk/index.php
ISSN 2707-9716 Print
ISSN 2707-9724 Online
https://doi.org/10.56520/asj.v5i3.312

Research Article

INDUCTION OF RESISTANCE IN MUNGBEAN AGAINST CERCOSPORA LEAF SPOT THROUGH PLANT DEFENSE ACTIVATORS

Laraib Malik[1], Muhammad Atiq[1]*, Nasir Ahmed Rajput[1], Muhammad Usman[1], Azeem Akram[1], Abdul Jabbar[2], Muhammad Noman Zahid[1], Waqas Ahmad[1], Muhammad Qasim[1], Wasi -ud- Din[2]

[1]*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*
[2]*Department of Agronomy, PARS Campus, Community College, University of Agriculture Faisalabad, Pakistan*
**Corresponding author:* muhammad.atiq@uaf.edu.pk

Abstract

Mungbean (Vigna radiata L. Wilczek), is a popular short-duration legume crop which is mostly cultivated in South and Southeast Asian countries. It is a rich source of proteins, carbohydrates, fats, minerals, and fibers. As an important source of dietary protein, it is usually consumed as whole seed or flour, or as sprouts. Cercospora Leaf Spot of Mungbean (CLSM) is a destructive disease caused by Cercospora canescens, which affects the whole crop, and causing 95% of yield losses. To mitigate these yield losses, five plant defense activators dipotassium hydrogen phosphate (K2HPO4), salicylic acid, carboxylic acid, citric acid, and benzoic acid were used in current study with 3 different concentrations (0.25, 0.5, and 0.75%) in a field trial arranged in randomized complete block design. Our field experiments revealed salicylic acid treated plants with minimum disease incidence (21.798%) followed by citric acid (25.131%), dipotassium hydrogen phosphate (27.466%), benzoic acid (29.064%), and carboxylic acid (35.043%) compared to untreated check. This revelation suggested the salicylic acid as a potent antifungal agent by activating the Mungbean defense systems for the management of CLSM.

Keywords: *Vigna radiata, Cercospora canescens, In-vivo evaluation, Salicylic acid, CLSM.*
(Received: 10-Sep-2023 Accepted: 10-Dec-2023) Cite as: Malik. L., Atiq. M., Rajput. N. A., Usman. M., Akram. A., Jabbar. A., Zahid. M. N., Ahmad. W., Qasim. M., Din. W. U., 2023 Induction of resistance in mungbean against cercospora leaf spot through plant defense activators. Agric. Sci. J. 5(3): 28-34.

1. INTRODUCTION

Mungbean (Vigna radiata L.) or green gram is a short-duration legume crop belongs to Fabaceae family. It was firstly originated from Dehli, India (Kumar et al., 2020, Rehman et al., 2021). Mungbean is widely cultivated across the world. It is mainly grown in India, Pakistan, Myanmar, Sri Lanka, China, Bangladesh, Thailand, Indonesia, and Australia (Khan et al., 2020).Total area of mungbean under cultivation is 7 million hectares with 721 kg/ha of average production. While in Pakistan, it is cultivated on an area of 231000 thousand hectares with 204.5 million tonnes production (Nair and Schreinemachers, 2020, GOP, 2021). Mungbean is a highly nutritive crop. Its seeds are a good source of carbohydrates (58.2%), protein (23.6%), minerals, (4%), fats (1.2%), and fibers (3.3%) (Chavhan et al.,2018). Mungbean sprouts consist of high amounts of niacin, ascorbic acid, and thiamine. It contains polyphenols that are important source of lipid metabolism, antioxidant, anti-inflammatory, and anti-diabetic. Due to rich in nutrition and antioxidant and hypocholesterolemic activities, it is used in the treatment of degenerative diseases, such as diabetes, cardiovascular and cancer (Kalim et al., 2021).

Mungbean is exposed to various diseases, such as Cercospora Leaf Spot of Mungbean (CLSM), powdery mildew, dry root rot, anthracnose, halo blight, yellow mosaic, and insect pests, especially pod borers, whitefly, aphids, thrips, and bruchids. Among all the constraints faced by mungbean, CLSM is one of the lethal biotic

stress caused by Cercospora canescens, which is responsible for 95% of yield losses under favorable environmental conditions (Abbas et al., 2020). It is a foliar disease and first time reported from Delhi, India (Munjal et al., 1960). Epidemiological conditions for the development of this disease require (25-30◦C) temperature and (90-100%) relative humidity (Shahbaz et al., 2014).

Commercial agriculture mainly depends on the application of chemicals to protect plants against pathogens by killing or suppressing their spores and cells growth. The use of synthetic pesticides on large scale cause resistance to pathogen and several harmful effects on the environment, humans, soil microbes, and animal health (Khan et al., 2022). The chemical compounds can also affect the growth, photosynthetic pigments, and reproductive organs of the plants by changing physiological and metabolic activities (Roberto et al., 2019). Recently, plant defense activators have been introduced to suppress the plant pathogens which have no toxic effects on plant health. Various signalling substances are involved in plant's defense system, which lead to the formation of defense-related compounds. These mechanisms are strongly associated to Ethylene (ET), Salicylic acid (SA), Jasmonic acid (JA), and Abscisic acid (ABA) (Vinod et al., 2018). Plant activators have no direct antibacterial or antifungal activity so they cannot produce resistance in pathogen (Ali et al., 2014). Keeping in view the importance of CLSM and its impact on moongbean, current study was performed by using different plant defense activators (Dipotassium hydrogen phosphate, SA, Carboxylic acid, Benzoic acid, and Citric acid) to check their efficacy against CLSM under field conditions.

2. Materials and Methods

2.1. Isolation of *C. canescens*

Potato dextrose agar (PDA) medium was prepared to isolate the fungus *C. canescens*. To create a 1000 ml volume, 250 g of peeled potatoes were boiled in distilled water to extract starch. Subsequently, 20 g of dextrose and 20 g of agar were added to the obtained starch. PDA media was autoclaved (RTA85) for 15 minutes at 121˚C temperature and 15 psi pressure. Streptomycin sulphate was added to autoclaved PDA media when it had been cooled to 50˚C to avoid bacterial growth. Samples were washed with tap water and were cut around the 5mm lesions with diseased and some healthy portion. Samples were treated with 1% sodium hypochlorite (NaOCl) for 10 seconds for sterilization and then wash with three repetitions of distilled water to remove the toxicity of NaOCl. After that, PDA was poured into the petri plates, and sterilized leaves portions were transferred to the solidified media plates for fungal growth and incubated these plates at 25˚C.

2.2. Purification and preservation

The pathogen was purified by single hyphal tip technique (Kouadri et al., 2021). The fungal mycelium was transferred with the help of a sterilized needle onto additional PDA plates to purify the pathogen culture (Leyronas et al., 2012; Atiq et al., 2021) and incubated at 25˚C for 10-15 days. For preservation of fungal culture, PDA was prepared and poured into a test tube in a slant position to increase surface area for fungal growth. Streptomycin sulphate was added to the media to avoid bacterial contamination. The pure culture was transferred to a test tube and incubated at 25˚C for 10-15 days. The test tubes were stored at -4˚C to enhance their maximum preservation time.

2.3. Pathogenicity test

For pathogenicity test, conidial suspension 5×10^5 conidia/ml of H_2O was prepared (Shahbaz et al., 2014). A hemocytometer was used to adjust the spore concentration (Wanjiku et al., 2020). Susceptible plant varieties such as MUNG, PML 18007 and PML 2005 were inoculated with conidial suspension by using foliar hand sprayer method (Arun et al., 2023).

2.4. Evaluation of plant defense activators under field conditions

Plant defense activators were assessed to determine their antifungal potential against *C. canescens* under field conditions. For this purpose, mungbean seeds were sown in field under Randomized Complete Block Design (RCBD). Five plant defense activators, Dipotassium hydrogen phosphate (K_2HPO_4), SA, Carboxylic acid, Citric acid, and Benzoic acid were used to manage the CLSM. Three different concentrations (0.25, 0.5, and 0.75%) of each plant defense activator were applied. The plant defense activators of 0.25g, 0.5g, and 0.75g were dissolved separately in 100mL of distilled water to make the 0.25%, 0.5%, and 0.75% solutions, respectively. For inoculum preparation, 24-48h old fungal culture was multiplied on sterilized mungbean grains. The fungal spore suspension of 5×10^5 conidia/mL of water was prepared. The hemocytometer was used to adjust the spore concentration (Wanjiku et al., 2020). Pathogen suspension was inoculated by using spraying method (Shahbaz et al., 2014). After 7 days of the appearance of disease symptoms, plant defense activators were applied by using hand sprayer. Disease incidence was recorded after 7, 14, and 21 days of treatment application by using disease incidence formula (Atiq et al., 2021).

Disease Incidence (%) = [(No. of infected plants) / (No. of total observed plants)] × 100

2.5. Data Analysis

The Least Significant Difference (LSD) was used to determine the significant differences between multiple treatments statistically at 5% probability level (Steel and Torrie, 1997).

3. Results:

3.1. Pathogenicity Test

Pathogenicity test revealed that pathogen produces similar symptoms on plants leaves after 6 days of inoculation. *C. canescens* growth was observed after re-isolation from infected plant tissues and matched with original pathogen. Both showed same results and proved Koch's postulates. Leaf spots appeared with reddish brown margins (2-4 mm and maximum10 mm in diameter) with yellow halos. Plants also exhibited angular water-soaked lesions on leaves which were grey to brown in color. The pathogen produced needle-like spores also which caused wrinkling of leaves, and reduced pod size.

Among all the treatments, SA expressed the minimum disease incidence (21.79%) followed by Citric acid (25.13%), K_2HPO_4 (27.46%), Benzoic acid (29.06%), and Carboxylic acid (35.04%) (Table 1). The interaction between treatment and concentration (T×C) exhibited that Salicylic acid showed lowest disease incidence (25.75, 22.25, 17.39%) followed by Citric acid (28.18, 25.77, 20.43%), K_2HPO_4 (31.11, 27.12, 24.15%), Benzoic acid (32.28, 28.80, 26.11%), and Carboxylic acid (38.45, 35.52, 31.15%) at 0.25, 0.50, and 0.75% concentrations, respectively (Table2, Figure 1). The interaction between treatments and various time intervals (Tr×T) revealed that SA exhibited minimum disease incidence (25.33, 22.19, 17.86%) followed by Citric acid (28.76, 25.21, 21.41%), K_2HPO_4 (25.33, 22.19, 17.86%) Benzoic acid (32.59, 29.31, 25.28%), and Carboxylic acid (38.20, 34.93, 31.10%) after 7, 14, and 21days intervals, respectively (Table 3, Figure 2).

Table 1. Effect of treatments on disease incidence of CLSM.

Treatments	**Disease incidence (%)**
Salicylic acid ($C_7H_6O_3$)	21.798e
Citric acid ($C_6H_8O_7$)	25.131d
Dipotassium hydrogen phosphate (K_2HPO_4)	27.466cd
Benzoic acid ($C_7H_6O_2$)	29.064c
Carboxylic acid (RCOOH)	35.043b
Control	58.776a
LSD	2.4559

Table 2. Interaction between treatments and concentrations (T×C) against CLSM under field conditions.

Treatments	Disease Incidence (%)		
	Concentrations (%)		
	0.25	0.50	0.75
Salicylic acid	25.750ghi	22.250ij	17.393k
Citric acid	29.183efg	25.778ghi	20.433jk
K2HPO4	31.117ef	27.125fgh	24.156hij
Benzoic acid	32.280de	28.801efg	26.111ghi
Carboxylic acid	38.456c	35.522cd	31.151ef
Control	55.500b	58.439ab	62.389a
LSD	4.2537		

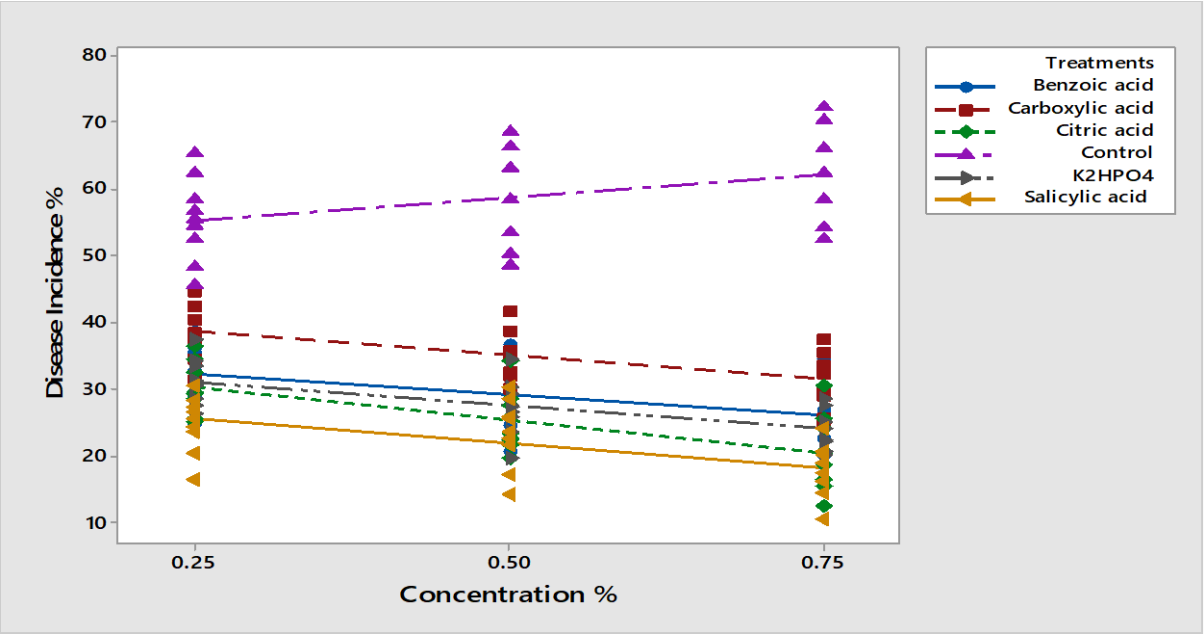

Figure 1. Interaction between treatments and concentrations (T×C)

Table 3. Interaction between treatments and different time intervals (Tr×T) against CLSM under field conditions.

Treatments	Disease Incidence (%)		
	Days		
	7	14	21
Salicylic acid	25.332ghi	22.194i	17.867j
Citric acid	28.767efgh	25.211ghi	21.417ij
K2HPO4	25.332ghi	22.194i	17.867j
Benzoic acid	32.596de	29.311efg	25.286ghi
Carboxylic acid	38.201c	34.933cd	31.994de
Control	54.800b	58.778ab	62.750a
LSD	4.2537		

4. Discussion

Mungbean cultivation faces significant challenges in the form of various diseases, insect pests, and abiotic stresses. CLSM caused by *C. canescens* is responsible for 95% of yield losses under favorable environmental conditions (Abbas et al., 2020). *C. canescens* produces a toxin known as perylenequinone-based cercosporin, which significantly contributes to the severity of the disease, and affects microbes, plants, and animals.

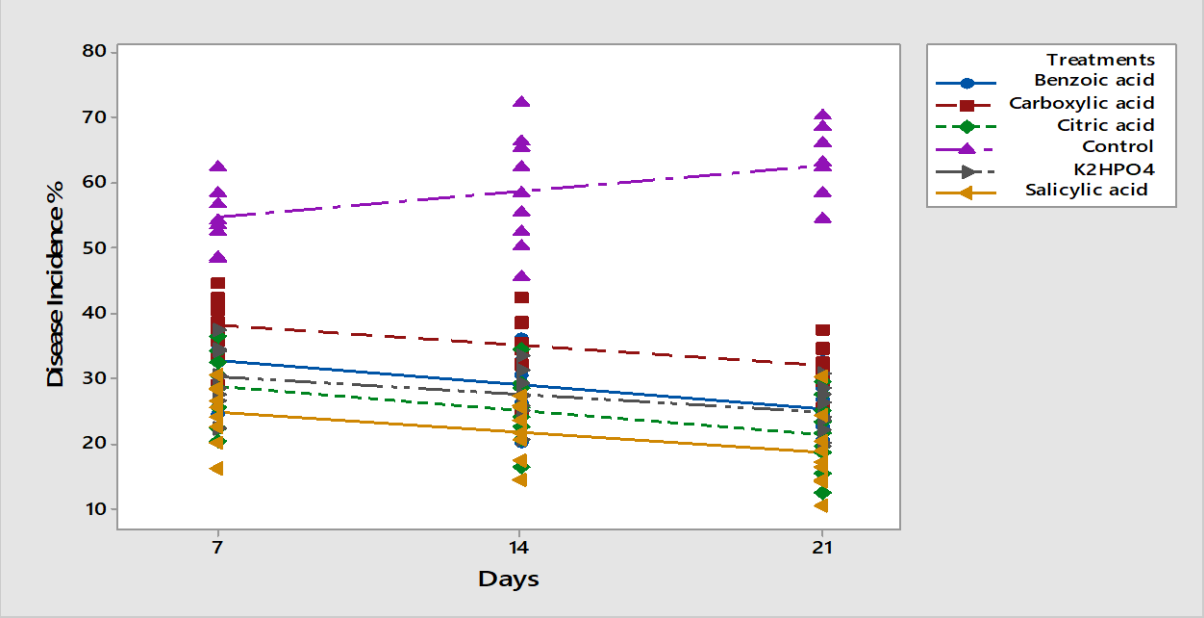

Figure 2. Interaction between treatments and time intervals (Tr×T)

Cercosporin produces reactive oxygen species (ROS) in plant tissues that destroy the host plants membrane and provide nutrients to support the growth of this intercellular pathogen (Daub and Ehrenshaft, 2000). On the first recognition of a pathogen attack, plants experience a short-term increase in ROS which include hydrogen peroxide (H2O2), superoxide (O-2), and hydroxyl (OH-) radicals. The ROS are considered to be involved in signal transduction, restrict pathogen entry, cause tissue necrosis, and initiate systemic acquired resistance (SAR) in plants. ROS are produced in plants as a result of pathogen attack through natural immune mechanisms to combat the pathogenic infection (Liu et al., 2020).

Various signalling substances are involved in plants defense system, which lead to the formation of defense-related compounds. These mechanisms are strongly associated to ET, SA, JA and ABA pathways (Vinod et al., 2018), and are essential components of the plant defense mechanisms against biotrophic pathogen infection. This is accomplished by a process known as SAR which is operated from infected parts of plants and sends signals to the healthy parts of plants to activate the pathogenesis-related genes. Plant activators have no toxic effect on the plant parts (Ali et al., 2014). In the present study, five plant defense activators including SA, K_2HPO_4, Benzoic acid, Carboxylic acid, and Citric acid were used. It was found that SA and K_2HPO_4 significantly inhibited disease incidence at high concentration compared to other treatments. Our findings are supported by the study of Atiq et al. (2021), they used five plant defense activators including CaCl2, Alpha-Tocopherol, Benzoic acid, SA, and K_2HPO_4 against a soil born fungal pathogens. Among all these treatments, they found K_2HPO_4 and SA significantly controlled more disease incidence. In another study, SA, acibenzolar-S-methyl, K_2HPO_4 and 2, 6-dichloro-isonicotinic acid were employed to assess their efficacy in managing anthracnose disease in cashew plants. SA showed good results at 5mM concentration (Lopez and Lucas, 2002). Zehra et al. (2017) used methyl Jasmonate and SA in their study against *Fusarium oxysporum* f.sp. *lycopersici*, which causes wilt disease in tomato, and found that the combined application of methyl Jasmonate and SA exhibited best results and expressed significant increase in phenolics and defense related proteins. The pathogen cannot produce resistance against plant activators because plant activators have no direct antibacterial or antifungal activity (Ali et al., 2014). SA is a key signalling

molecule in plant defense against pathogens and plays a vital role in activating the plant defense response. SA signalling pathways are known to be involved in ROS production, regulation of pathogenesis-related proteins, synthesis of phytohormones (JA and ET) (Boamah et al., 2023). The recent studies demonstrated that SA is an essential hormone for plant immunity, HR response, and resistance to biotic invasion in local and systemic tissues. It is also essential for the modification of plants reactions for their defense to various abiotic stresses such as cold, heat, and drought (Kang et al., 2014).

5. Conclusion

In field experiment, SA showed significant results and expressed minimum disease incidence followed by citric acid, dipotassium hydrogen phosphate (K_2HPO_4), benzoic acid, and carboxylic acid. It was found that SA showed significant results at 0.75% concentration and provided valuable insights for the effective management of CLSM.

6. Conflict of Interest

The authors have declared the absence of any conflicting interests.

7. REFERENCES

Abbas, H., M.A. Iqbal, M. Kamran, M.U. Shahbaz, H.U. Kamber, N. Javed, M. Junaid, H. Abbas and M.E. Ul-Haq. 2020. Evaluation of advanced mung bean germplasm against Cercospora leaf spot and its in-vitro management by different fungicides. Pak. J. Agric. Res. 33(4): 231-872.

Ali, Z., M. Shah, A. Nawaz, M. Shahjahan, H. Butt, M. Shahid and R. Ahmed. 2014. Assessment of induced systemic resistance through antagonistic rhizobacterial potential with salicylic acid against karnal bunt of wheat. Pak. J. Phytopathol. 26(2): 253-258.

Arun, A., V. Ambethgar, A. Kalyanasundaram, M. Loganathan, K. Senthil, and A. Mohanasundaram. 2023. Unleashing the potential of endophytic Beauveria bassiana as a biocontrol agent against rice leaf folder (*Cnaphalocrocis medinalis*). Pharma Innov. J. 12(8):2136-2141.

Atiq, M., S. Adil, N.A. Rajput, S.T. Sahi, M. Usman, S. Iqbal, S.A. Chand, A. Nawaz, A.M. Arif and A. Akram. 2021. Activation of *Capsicum annuum* L. Defense System against Fusarium Wilt through Plant Activators and their Impact on Horticultural Attributes. Int. J. Plant. Pathol. 10(3): 155-166.

Boamah, S., T. Ojangba, S. Zhang, N. Zhu, R. Osei, R. John Tiika, and B. Xu. 2023. Evaluation of salicylic acid (SA) signaling pathways and molecular markers in *Trichoderma*-treated plants under salinity and Fusarium stresses. A Review. Eur. J. Plant Pathol. 166(3), 259-274.

Chavhan, S., M. Darade and V. Devarkar. 2018. Mycopathological Studies on *Vigna radiata* (L.) Wilczek (Green gram) from Patur, Dist. Akola (MS), India. Plantae. Scientia. 1(3): 55-59.

Daub, M.E., and M. Ehrenshaft. 2000. The photoactivated Cercospora toxin cercosporin: contributions to plant disease and fundamental biology. Annu. Rev. Phytopathol. 38(1): 461-490.

GOP. 2021. Economic survey of Pakistan. Islamabad: Economic Advisor's Wing Ministry of Finance. 20.

Kalim, A., M. Zaheer, M.U.A. Siddiqui, S. Ahmed and M.M. Hassan. 2021. Nutritional value, Ethnomedicine, Phytochemistry and pharmacology of *Vigna radiata* (L.) R. Wilczek. J. Pharmacogn. Phytochem. 10(2): 54-58.

Kang, G., G. Li and T. Guo. 2014. Molecular mechanism of salicylic acid-induced abiotic stress tolerance in higher plants. Acta Physiol. Plant. 36, 2287-2297.

Khan, B.A., A. Hussain, A. Elahi, M. Adnan, M.M. Amin, M.D. Toor, A. Aziz, M.K. Sohail, A. Wahab and R. Ahmad. 2020. Effect of phosphorus on growth, yield and quality of soybean (Glycine max L.); a review. Int. J. Appl. Res. 6(7): 540-545.

Khan, M.R., Z.A. Siddiqui and X. Fang. 2022. Potential of metal and metal oxide nanoparticles in plant disease diagnostics and management: Recent advances and challenges. Chemosphere. 297: 134114.

Kouadri, M.E.A., A.A. Bekkar and S. Zaim. 2021. First report of Bipolaris sorokiniana causing spot blotch of lentil in Algeria. New Dis. Rep. 43: e12009.

Kumar, N., S. Kumar, S. Prajapati and S. Maurya. 2020. Cercospora leaf spot disease of green gram and its management: A review. J. Pharmacog. Phytochem. 9(1): 1574-1576.

Leyronas, C., M. Duffaud and P.C. Nicot. 2012. Compared efficiency of the isolation methods for Botrytis cinerea. Mycol. 3(4), 221-225.

Liu, F., M.G. Saavedra, J.A. Champion, K.K. Griendling and N.L. Ng. 2020. Prominent contribution of hydrogen peroxide to intracellular reactive oxygen species generated upon exposure to naphthalene secondary organic aerosols. Environ. Sci. Technol. Lett. 7(3): 171-177.

Lopez, A. and J. Lucas. 2002. Effects of plant defence activators on anthracnose disease of cashew. Eur. J. Plant. Pathol. 108: 409-420.

Munjal, R., G. Lall and B. Chona. 1960. Some Cercospora species from India.-VI. Ind. Phytopathol. 14: 179-190.

Nair, R. and P. Schreinemachers. 2020. Global Status and Economic Importance of Mungbean. In: Nair, R., Schafleitner, R., Lee, SH. (eds) The Mungbean Genome. Compendium of Plant Genomes. Springer. Pp. 1-8.

Rehman, A.U., M.E. Khan, K.P. Akhtar, S. Kaukab, S. Saeed, M.J. Asghar and J. Salim. 2021. Identification of Sources of Disease Resistance to Mungbean *Yellow Mosaic Virus (Mymv)* and Cercospora Leaf Spot Disease (Cls) in Mungbean (Vigna Radiata L.). Pak. J. Phytopathol. 33(2):335-347

Roberto, S.R., K. Youssef, A.F. Hashim and A. Ippolito. 2019. Nanomaterials as alternative control means against postharvest diseases in fruit crops. Nanomater. Basel. 9 (12): 17-52.

Shahbaz, M.U., M.A. Iqbal, M. Rafiq, A. Batool and M. Kamran. 2014. Efficacy of different protective fungicides against Cercospora leaf spot of mungbean (Vigna radiata l. Wilczek). Pak. J. Phytopathol. 26(2): 187-191.

Steel, R.G.D. and J.H. Torrie. 1997. Principles and procedures of statistics. Principles and procedures of statistics.

Vinod, K. and A. Sabah. 2018. Plant defense against pathogens: the role of salicylic acid. Res. J. Biotechnol. 13(12): 97-103.

Wanjiku, E.K., J.W. Waceke, B.W. Wanjala and J.N. Mbaka. 2020. Identification and pathogenicity of fungal pathogens associated with stem end rots of avocado fruits in Kenya. Int. J. Microbiol.

Zehra, A., M. Meena, M.K. Dubey, M. Aamir and R.S. Upadhyay. 2017. Activation of defense response in tomato against Fusarium wilt disease triggered by *Trichoderma harzianum* supplemented with exogenous chemical inducers (SA and MeJA). Braz. J. Bot. 40, 651-664