www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 836-841

www.thepharmajournal.com
Received: 01-10-2021
Accepted: 03-11-2021

Pravallika Sree Rayanoothala
Department of Plant Pathology, Bidhan Chandra Krishiviswavidyalaya, Mohanpur, Nadia, West Bengal, India

Sunita Mahapatra
Department of Plant Pathology, Bidhan Chandra Krishiviswavidyalaya, Mohanpur, Nadia, West Bengal, India

Srikanta Das
Department of Plant Pathology, Bidhan Chandra Krishiviswavidyalaya, Mohanpur, Nadia, West Bengal, India

Corresponding Author
Pravallika Sree Rayanoothala
Department of Plant Pathology, Bidhan Chandra Krishiviswavidyalaya, Mohanpur, Nadia, West Bengal, India

Accumulation of total phenols and ortho-dihydroxy phenols and activity of a potential oxidant scavenger: ascorbic acid in selected mungbean genotypes against *Macrophomina phaseolina*

Pravallika Sree Rayanoothala, Sunita Mahapatra and Srikanta Das

Abstract
The most important legume crop in the world is mungbean (*Vigna radiate* L. Wilzeck). Vigna is a member of the Papilinoidae subfamily and Leguminoseae family. It is primarily grown in Asia, but cultivation has recently spread to Africa and the Americas. *Vigna radiata*, on the other hand, is consumed in the form of sprouts and dry seeds due to its high protein content. Mungbean is a valuable crop that is commonly produced in dry and semiarid environments due to its rapid growth and early maturity properties, as well as its capacity to replenish soil fertility. The fundamental feature of mungbean is that it reduces fertiliser use and provides nitrogen fertiliser to agriculture fields in short supply, strengthening soil structure and giving plant protein, but flowering and maturity times are shortened under stress compared to well-watered circumstances. Crop yields are severely reduced due to irregular annual rainfall and a lack of source management.
Charcoal rot being a major concern on mungbean whose control largely depends on the application of chemical pesticides however it is not the long term solution due to environment concern and risk due to fungicide residues. Under these circumstances, Induced resistance is one of the most dominant mechanism in managing the disease by increasing the activity of various defense related enzymes and non-enzymatic antioxidants. Role of inducers *viz.* Salicyclic acid (SA), Jasmonic acid (JA) at three different concentrations *viz.* low, medium and high concentrations, *viz*. JA (1mM, 2.5 mM, 4 mM) & SA (0.5 mM, 1 mM, 2 mM) were evaluated on induction of resistance to manage Charcoal rot of mungbean in three different varieties *viz.* resistant, moderately susceptible and susceptible against the disease was studied in net house. Elicitor treatments exhibited maximum content of total phenols, Ascorbic acid content and Orthodihydroxy phenols (OD- Phenols) content as compared to water sprayed control on un-inoculated plants. Among all the treatments, SA@2 mM was most effective on increasing the observed parameters as total phenol content Ascorbic acid content and Orthodihydroxy phenols (OD- Phenols) content followed by JA on disease resistance. Lower amount of phenols and low accumulation of OD-phenols were recorded in healthy plants when compared with infected plants. These changes can be attributed to the role played by inducers and it is well known fact that used elicitors played an important role in enhancing the defense mechanism in plants.

Keywords: elicitors, biochemical changes, charcoal rot, mungbean, pot experiment

Introduction
In our country, mungbean is an important pulse crop. Mungbean is grown in three different seasons in India. Kharif, Rabi, and Summer are the three seasons. In the eastern and southern parts of India, it is also grown in rainfed conditions during kharif and residual moisture throughout rabi. In the kharif season, the seed rate of mungbean is 10-15 kg/ha, while in the spring season, the seed rate is 20-3 kg/ha (Chadha, 2010) [7]. Mungbean is high in polyphenolic chemicals, such as simple phenols, flavonoids, and tannins, all of which are natural antioxidants (Prior and Gu, 2005; Sanos Bulega and Scalbert, 2000; Amarowicz *et al*., 2004 and Troszunska and Cisa, 2002) [19, 21, 2, 24]. However, biotic variables are to blame for up to 44% -60% of pulse crop losses (Deshkar *et al*., 1974; Bashir and Malik, 1988) [10, 3]. *Macrophomina phaseolina* causes mungbean charcoal rot, which reduces crop yield, especially in arid areas (Charles 1978; Hoes., 1985) [8, 15].
Macrophomina phaseolina affects the root, stem, branches, petiole, leaves, pods, and seeds of plants. Furthermore, *Macrophomina phaseolina* seed infection varies from 2.2-15.7 percent, resulting in a 10.8 percent loss in grain yield and a 12.3% fall in protein content in urdbean (Kaushik *et al*., 1987) [16].

Macrophomina phaseolina causes red to brown lesions on roots and stems in mature plants. Plants became defoliated and wilted as a result of the dark mycelia and black microscelerotia (Abawl and Pastor- Corrales, 1990) [1]. In the temperature range of 60-65 °C, *Macrophomina phaseolina* is a heat tolerant pathogen (Bega and Smith, 1962: Milhail and Acron, 1984) [4]. Charcoal rot is produced by a common soil-borne fungus called *Macrophomina phaseolina* (Whittaker, 1969) [25] in its imperfect form, and Sclerotium bataticulum Taub in its perfect state (Butl.). The Botryosphaeriaceae family includes this fungus. It infects almost 500 plant species in 75 families across a large geographic range (Dhingra and Sinclair, 1978; Bouhot, 1967, 1968 and Gray et. al, 1990; Crous *et al*, 2006) [11, 6, 14, 9]. Seedling blight, stem rot, and pod rot are all caused by *Macrophomina phaseolina* (Sinclair, 1982) [23]. Plant mortality or lodging cause yield losses caused by *Macrophomina phaseolina*. Lodging happens when the stem becomes weak and microsclerotia form in the vascular tissues (Edmunds, 1964; Odvody and Duke, 1979) [13, 18]. Charcoal rot has resulted in a 60% reduction in production (Steven *et al*., 1987). The infection of Charcoal rot causes a 330-50 percent annual loss of mungbean (Ramazami et. al., 2007; Senthil Umar *et al*., 2009) [20, 22]. With the steady supply of highly effective and newer broad spectrum fungicides over the past decades, indiscriminate constant application of various chemicals has become a major alternative to developing resistant cultivars, biological control, cultural practises like crop protection, and use of chemicals has become the most important component of disease management strategy in mungbean. With the steady supply of highly effective and newer broad spectrum fungicides over the past decades, indiscriminate constant application of various chemicals has become the most important component of disease management strategy in mungbean As a result of these circumstances, the use of environmentally acceptable elicitors in plant disease management has gained prominence and attention. Elicitors are chemicals that activate chemical defence in plants at low concentrations; they operate as signal compounds that inform the plant about when to engage chemical defence. (Ebel and Cosio, 1994; Boller, 1995) [12]. The present study reports phenolic accumulation, Ortho-dihydroxy phenols and activity of a potential oxidant scavenger: ascorbic acid selected mungbean genotypes against *Macrophomina phaseolina* The experiment reports the resistance, moderately susceptible and susceptibility reactions in plant were might be attributed by the differential metabolomics responses of the plant. Where, in particular, the resistance reaction was mainly because of elevated defense metabolites in plant.

Materials and Methods

Plant materials and pathogen inoculation

The seeds of three genotypes of mungbean (*Vigna radiata*) *viz*., resistant, moderately susceptible and susceptible *viz.*, Bireswar, 2-sukumar 3 and Samrat respectively were surface sterilised by 1.0% sodium hypochlorite and sown post seed treatment with various elicitors with different concentrations *viz*. JA (1mM, 2.5 mM, 4 mM); SA (0.5 mM, 1 mM, 2 mM) as done by Biswas *et al*., Isolations of the infected stems and leaves with typical symptoms were made. To eliminate soil, the stems and leaves were thoroughly rinsed with tap water. Infected plant portions were cut into small pieces (0.5-1.0 mm) and surface sterilised for 2 minutes with a 0.1 percent mercuric chloride solution, then washed three times with sterilised glass distilled water and blot dried. Each potato dextrose agar (PDA) slant received one bit aseptically. After that, the pathogen was grown in culture tubes for four to five days at 30 ± 1 ºC.

Mungbean plants of a specified genotype (15 days old) were infected using a mycelial suspension of 3 days old culture produced on potato dextrose broth that was filtered and homogenised to give $1X10^3$ viable propagules per millilitre for the pot experiment. This suspension was sprayed on the mungbean plants' foliage with a hand atomizer until it ran off. After keeping the inoculated seedlings at high humidity for 150 hours, diseased samples were obtained to determine phenol accumulation, Ortho-dihydroxy phenols and total ascorbic acid. Control plants were raised and sprayed with distilled water.

Treatment details

T1: Seed treatment with elicitors, with pathogen inoculation
T2: Seed treatment with elicitors, without pathogen inoculation
T3: Without seed treatment (water), with pathogen inoculation
T4: Without seed treatment (water), without pathogen inoculation

Biochemical studies

Mungbean seedlings were collected from different treatments and the phenol accumulation, Ortho-dihydroxy phenols and total ascorbic acid content of were estimated at 15 DAS, 18 DAS and 5 DAI.

Determination of total phenols

Folin-Ciocalteau Reagent was used to calculate total phenols (Sadasivam and Manickam, 1992) [26]. A sample of 0.5 g from each replicate sample was pulverised in a mortar and pestle with ten times the volume of 80 percent ethanol and centrifuged at 10,000 rpm for 20 minutes. Total phenols were measured colorimetrically using Folin-Ciocalteu reagent using a sample of 20 l. Gallic acid was used as a reference and the absorbance was measured at 650 nm against a reagent blank.

Determination of total ascorbic acid

The determination of ascorbic acid content was done according to spectrophotometric method given by Davis and Masten (1991).

Determination of Ortho-dihydroxy phenols

Mahadevan and Sridhar's approach was used to calculate o-dihydroxy phenols (1986). The estimation was done with 200 l of aliquot and Arnow's reagent. The absorbance was measured using catechol as a reference at 515 nm.

Results and Discussion

The effect of elicitors were investigated in terms of the phenol accumulation and total ascorbic acid content three genotypes of mungbean (*Vigna radiata*) *viz*., resistant, moderately susceptible and susceptible *viz.*, Bireswar, 2-sukumar 3 and Samrat respectively against charcoal rot caused by *Macrophomina phaseolina*. Elicitors used at different concentrations reduced the disease incidence and significantly showed the difference among the observed defense related compounds (Table 1-2 and Fig.1-2). It is observed that with increase in concentration of the different elicitors showed a

significant increase in defense related compounds both in pathogen and without pathogen inoculated plants and their differences were significant in all the three genotypes.

Table 1: Impact of various elicitors on mungbean genotypes on total Phenols content

Treatments	Total Phenols (mg/g dry weight)								
	Bireswar (Resistant)			2- sukumar 3 (Moderately susceptible)			Samrat(Susceptible)		
	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI
JA @ 1 mM	6.89	8.98	11.66	12.78	15.02	19.68	14.84	16.97	20.42
JA @2.5 mM	6.98	9.40	11.81	15.24	17.48	18.05	14.93	17.07	21.88
JA @ 4 mM	7.40	11.10	9.93	15.85	18.09	19.96	16.63	18.77	23.15
SA @ 0.5 mM	6.03	8.03	11.18	13.52	15.77	18.53	14.75	16.89	20.50
SA @ 1 mM	7.75	9.75	12.80	13.62	15.87	19.02	16.22	18.35	20.60
SA @ 2 mM	8.32	10.32	13.08	14.31	16.56	20.15	17.49	19.63	21.51
CONTROL	5.92	5.27	6.45	9.52	11.76	12.00	13.19	15.32	13.93
SEM	0.34	0.32	0.35	0.59	0.59	0.36	0.53	0.56	0.51
CD	1.02	0.96	1.07	1.79	1.79	1.06	1.61	1.70	1.54
CV	8.04	5.74	5.22	7.21	6.22	3.16	5.83	5.39	4.12

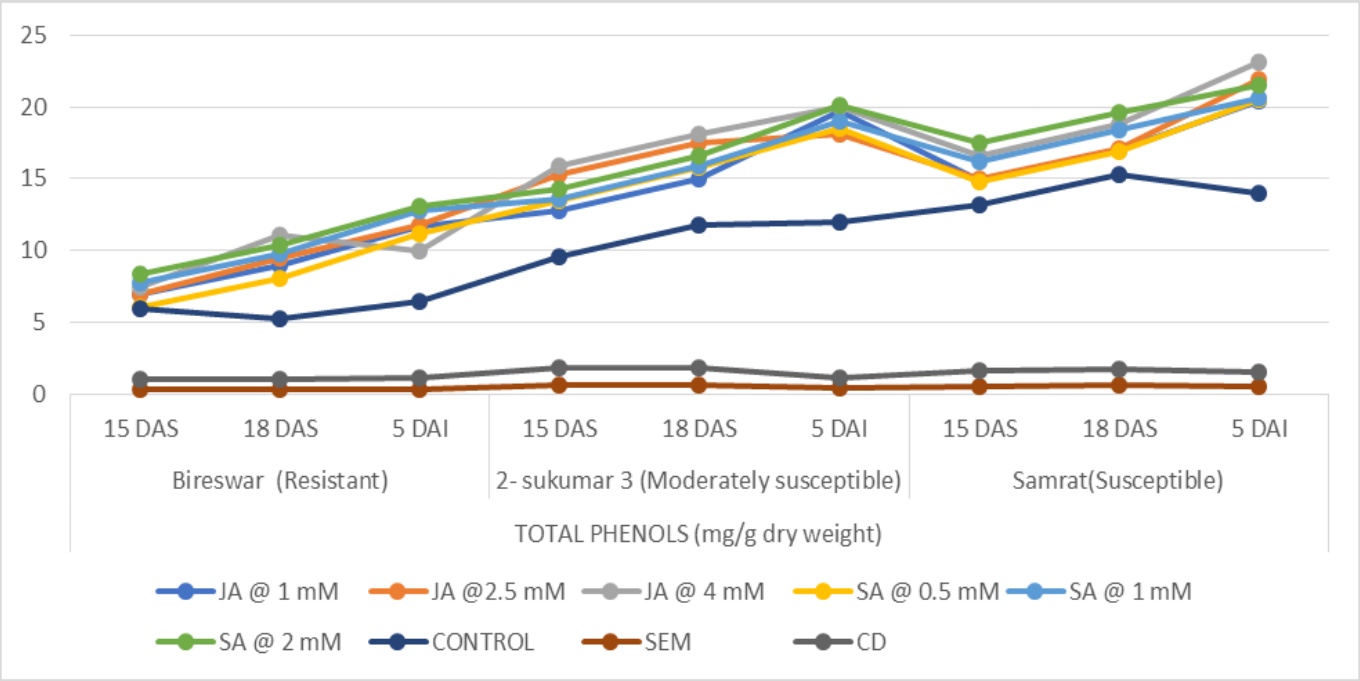

Fig 1: Graphical representation of impact of elicitors on total phenols in selected genotypes

It is evident from the, (Table 1 and Fig. 1) however, the accumulation of Phenols was maximum in Samrat mungbean genotype when compared to the other two genotypes *i.e*., SA@2 Mm 17.49 mg/g, SA@2 Mm 19.63 mg/g and SA@2 Mm 21.51 mg/g, when compared to SA@2 Mm 8.32 mg/g, JA@4mM 11.10 mg/g, SA@2 Mm 13.08 mg/g and JA @4Mm 15.85 mg/g, JA @4Mm 18.09 mg/g, SA@2 Mm 20.15 mg/g of Bireswar and 2-sukumar 3 genotypes at 15 DAS, 18 DAS and 5 DAI respectively Phenols plays a major role in conferring resistance to plants against infection by microbes by inactivation of fungal enzymes or viral nucleoproteins by accumulating in the infected tissue to inhibit the growth of the pathogens of the host and may be related to their release from glycosidic esters by enzymatic activity of host or pathogen (Meena *et al.*, 2014). These compounds have been correlated with the resistance of plants to infectious agents (Singh, 2000). It is evident from the Table 1 and Fig. 1, that accumulation of phenol compounds are maximum in diseased plants of susceptible mungbean genotype as compared to healthy resistant genotypes which were in accordance with the findings of Hrubcova *et al.* (1992), Ferraris *et al*. (1987).

It is evident from the table2, the total ascorbic acid content was maximum in Bireswar mungbean genotype when compared to the other two genotypes *i.e*., JA@4 Mm 101.47 mg/g, JA@4mM 109.01 mg/g, SA@0.5 Mm 111.89mg/g, when compared to JA@4 Mm 86.67 mg/g, SA@2 Mm 103.33 mg/g, JA@4mM 107.34mg/g and JA @4Mm 77.71 mg/g, JA @4Mm 81.59 mg/g, SA@0.5Mm 81.59 mg/g 2-sukumar 3 and Samrat genotypes at 15 DAS, 18 DAS and 5 DAI respectively. The Present study on non-enzymatic antioxidant revealed that the level of ascorbate was increased significantly over their respective uninoculated controls with the increasing stress period in all the varieties tested. Above findings are strongly supported by the report of Mallick *et al*. (2017). Ascorbic acid, acts as powerful antioxidant in tissues and an enhanced level has also been observed in stressed plants as a resistance index against the pathogen (Gupta *et al*., 2012). When the ROS level increases in plants that are exposed to stress, enhanced production of nonenzymatic antioxidants in plant cells like ascorbic acid will play a crucial role in minimizing ROS induced oxidative stress (Gill and Tuteja, 2010) and (Lu *et al.* 2019).

Table 2: Impact of various elicitors on mungbean genotypes on total ascorbic acid content

Treatments	Total ascorbic acid (mg/g fresh weight)								
	Bireswar (Resistant)			2- sukumar 3 (Moderately susceptible)			Samrat(Susceptible)		
	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI
JA @ 1 mM	90.48	98.02	83.23	79.29	96.27	98.62	69.06	72.93	62.46
JA @2.5 mM	95.94	100.14	100.90	77.89	95.87	98.22	72.85	76.72	72.93
JA @ 4 mM	101.47	109.01	106.35	86.67	98.32	107.34	77.71	81.59	76.72
SA @ 0.5 mM	75.07	79.27	111.89	78.02	98.00	100.36	59.41	61.62	81.59
SA @ 1 mM	81.07	85.28	85.48	77.50	97.81	100.17	63.33	67.20	63.29
SA @ 2 mM	91.71	95.91	91.49	77.92	103.33	105.69	72.81	76.68	67.20
CONTROL	43.72	57.93	102.12	71.69	95.90	98.25	38.82	42.69	76.68
SEM	3.51	3.68	3.57	2.19	2.78	2.93	2.64	2.38	2.62
CD	10.65	11.16	10.82	6.65	8.42	8.90	8.01	7.21	7.94
CV	6.81	6.74	6.40	4.78	4.95	5.06	6.62	5.66	6.42

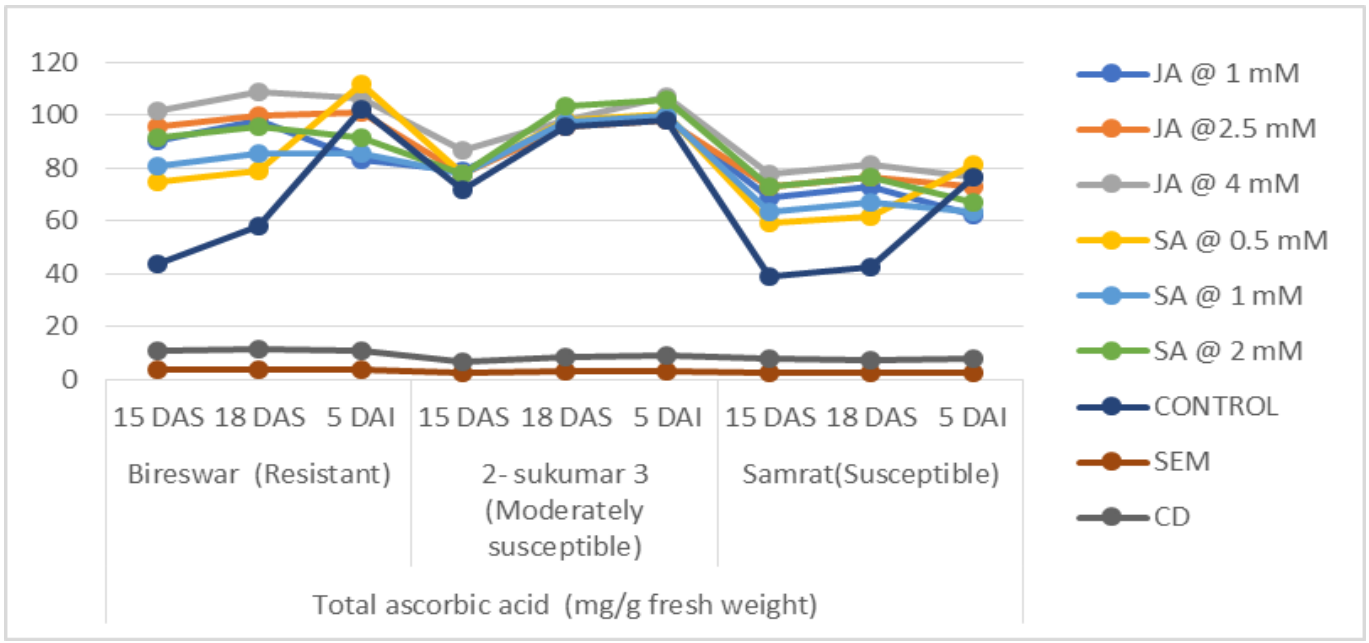

Fig 2: Graphical representation of impact of elicitors on ascorbic acid content in selected genotypes of mungbean

As total phenols, similar trend was followed by o-dihydroxy phenols content. Significant increase in O-dihydroxy phenols was observed with increase in concentrations of different inducers and with the age of the plant. Is has also been observed that increase in OD phenol content was still higher in inoculated when compared to uninoculated treatments. It is evident from the table3, the OD-Phenols content was maximum in Samrat mungbean genotype when compared to the other two genotypes *i.e.*, JA@1 Mm 2.42 mg/g, JA@4mM 2.27mg/g, JA@4mM 3.18mg/g, when compared to JA@4 Mm 1.03 mg/g, JA@2.5 Mm 1.11 mg/g, JA @2.5mM 1.42 mg/g and JA @4Mm 1.77 mg/g, JA @4Mm 2.19 mg/g, JA@4Mm 3.18 mg/g Bireswar and 2-sukumar 3 at 15 DAS, 18 DAS and 5 DAI respectively. It was observed that OD phenols increased with progress of infection and with increase in plant age. Ortho dihydroxy phenol concentration as a resistance factor because they become highly reactive upon oxidation and may form substances toxic to pathogens or inactivate enzymes including hydrolytic enzymes produced by plant pathogenic fungi. The oxidation of o-dihydroxy phenols in resistant plant varieties may stimulate the active defense reaction, while such reactions were less strong in susceptible genotypes (Mathpal *et al*., 2011).

Table 3: Impact of various elicitors on mungbean genotypes on OD- Phenols content

Treatments	Total Ortho-DI-Hydroxy Phenols (mg/g dry weight)								
	Bireswar (Resistant)			2- sukumar 3 (Moderately susceptible)			Samrat(Susceptible)		
	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI
JA @ 1 mM	0.96	1.05	1.26	1.39	1.53	1.99	2.42	2.20	2.58
JA @2.5 mM	1.02	1.11	1.36	1.42	1.60	1.95	2.15	2.26	2.44
JA @ 4 mM	1.03	1.05	1.33	1.50	1.77	2.19	2.16	2.27	3.18
SA @ 0.5 mM	0.67	0.83	0.97	1.23	1.44	1.60	1.83	2.01	2.08
SA @ 1 mM	0.71	0.70	1.11	1.14	1.28	1.64	1.99	2.14	2.13
SA @ 2 mM	0.72	0.76	1.02	1.12	1.33	1.66	1.89	1.96	2.14
CONTROL	0.63	0.71	0.93	1.03	1.20	1.56	1.75	1.86	2.04
SEM	0.03	0.06	0.03	0.04	0.07	0.05	0.12	0.09	0.17
CD	0.08	0.17	0.10	0.12	0.20	0.17	0.35	0.27	0.50
CV	5.63	10.61	4.81	5.12	7.79	5.17	9.69	7.28	11.88

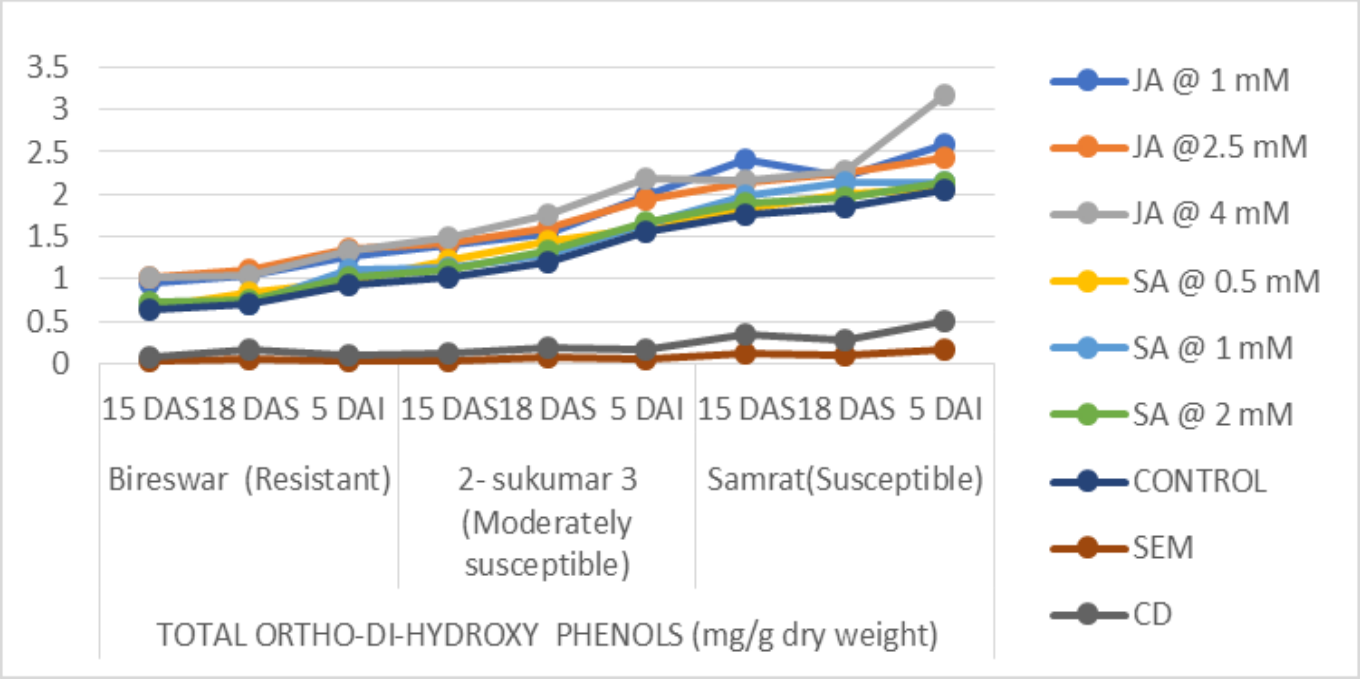

Fig 3: Graphical representation of impact of elicitors on OD- Phenols in selected genotypes of mungbean

Future scope of research

The Studies in this area may yield knowledge on host-pathogen interactions that can be used in resistance breeding, allowing a desirable feature to be generated by introducing resistance into a promising but susceptible mungbean genotype. At the gene level, mechanisms responsible for triggering defence genes via signal transduction that is activated by elicitors should be investigated. It is necessary to look for various inducers that are responsible for inducing resistance, which can be done using enzyme markers. The effectiveness of various elicitors in the field should be studied on different genotypes of the crop, and their performance can be assessed using resistance markers. Elicitors should be promoted by proposing them as a seed treatment in farmer's fields, as a potential alternative to chemical fungicides in the control of a few diseases where pathogen-induced fungicide resistance is a serious issue.

Conflict of interest

There are no conflict of interests to declare to publish this article.

Acknowledgements

The research assistance provided under Department of Plant Pathology and Department of Agricultural Biochemistry, BCKV is highly acknowledged.

References

1. Abawl GS, Pastor Corrales MA. Root rots of bean in Latin America and Africa: diagonsis, research, methodologies and management strategies, CIAT, Cali, Colombia, 1990, 114.
2. Amaowicz R, Troszynska A, Barylopiidia N, Shahidi F. Polyphenolics extracts from legume seeds; correlations between total antioxidant activity, total phenolics content, Tannins content and astringency. Journal of Food Lipids 2004;11:278-286.
3. Bashir M, Malik BA. Diseases of major pulse crops in Pakistan-A Review Tropical Pest Management 1988;34(3):309-314.
4. Bega, Smith. Time –Temperature relationships in thermal inactivation of sclerotia of *Macrophomina phaseolina*. Journal of Phytopathology 1962;52:632-635.
5. Boller T. Antimicrobial function of the plant hydrolases, chitinase and β-1,3-glucanase. In: Firtig B and Legrand M (eds) Mechanisms of Plant Defense *Responses*. Kluwer academic publishers, Netherlands 1993, 391-400.
6. Bouhot D. Étude du *Macrophomina phaseoli* sur arachide. Agronomic Tropic 1967;22:1165-1171.
7. Chadha ML. Short duration Mungbean: A new success in South Asia. Asia-Pacific Association of Agricultural Research Institutions, FAO Regional Office for Asia and Pacific Bangkok. Thailand 2010.
8. Charles YY. "Mungbean diseases and control, "In Proceedings of the Ist International Mungbean Symposium, AVRDC 1978.
9. Crous P., Slipper B, Wingfield MJ, Rheeder J, Maraas WFO. Phytogenetic lineages in the *Botryosphaeriaceae* studies in Mycology 2006;55:235-253.
10. Deshkar MV, Khare MN, Singh L. A Rhizoctonia disease for mungbean (*Phaseolus aureus Roxb*.) in Madhya Pradesh. J. N. Krishi Vishwa Vidhyalya Research Journal 1974;3:40-43.
11. Dhingra OD, Sinclair JB. An annotatal bibiliography of *Macrophomina phaseolina*. Brasil Universal Federal de Vicosa 1978;244:1905-1975.
12. Ebel J, Cosio EG. Elicitors of plant defense responses. Int Rev Cytol 1994;148:1-36.
13. Edmunds LK, Voigt RL, Carasso FM. Use of Arizona climate to induce charcoal rot in grain sorghum. Plant Disease Reports 1964;48:300-302.
14. Gray FA, Kolp BJ, Mohamed MA. A disease survey of crops grown in the Bay Region of Somalia, East Africa. FAO Plant Protection Buletin 1990;38:39-47.
15. Hoes JA. *Macrophomina phaseolina* causal agent of charcoal rot of sunflower and other crops. *Agriculture Canadian Research Station*, Modern Manitoba 1985.
16. Kaushik CD, Chand JN, Saryavir. Seedborne nature of *Rhizoctonia bataticola* causing leaf blight of mung bean. Indian Journal of Mycology and Plant Pathology

1987;17:154-157.
17. Mihail JD, Alcorn SM. x Effects of soil solarization on *Macrophomina phaseolina* and *Sclerotium rolfsii*. Plant Disease 1987;68:156-159.
18. Odvody GN, Dunkle LD. Charcoal stalk rot of sorghum: Effect of environment on host parasite relation. Phytopathology 1979;69:250-225.
19. Prior RL, Gu L. Occurrence and Biological significance of Pro antho cyanidins in the american diet. Phytochemistry 2005;66:2264-2280.
20. Ramezani M, Shier WT, Abbas HK, Tonos JL, Baird RE, Sciumbato GL. Soybean charcoal rot disease fungus *Macrophomin phaseolina* in Mississippi produces the phytotoxin(-)-botryodiplodin but no detectable phaseolinone. Journal of Natural Products 2007;70(1):128-129.
21. Santos Bulrga L, Scalbert A. Pro anthocyanidine and tannins like compounds- nature, occurrence and dietary intake and effect on nutrients and health. Journal of Science Food and Agriculture 2000;80:1094-1117.
22. Senthilkumar M, Swarnalakshmi K, Govindasamy V, Lee YK, Annapurna K. Biocontrol potential of soybean bacterial endophytes against charcoal rot fungus, *Rhizoctonia* bataticola. Current microbiology 2009;58(4):288-293.
23. Sinclair JB. Compendium of soybean diseases 2nd edition. American phytopathology Society. St. Paul MNP, 1982, 104.
24. Trosynska A, Cisa F. Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. Czech Journal of Food Science 2002;20:15-22.
25. Whittaker RH. “New concepts of kingdom or organisms evolutionary relations are better represented by new classifications by the traditonal two kingdom science”. 1969;163:150-194.
26. Sadasivam S, Manickam, A. Biochemical methods for agriculture sciences, Wiley Eastern Limited, New Delhi, 1992, 11-12.