Changes in Biochemical Constituents and Enzymatic Activity of Moth Bean Seeds due to Infection of Xanthomonas axonopodis pv. phaseoli

Vishal Sharma, Kailash Agrawal, Nanda Ram and Mukta Agrawal

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 04

Res. Jr. of Agril. Sci. (2022) 13: 1255–1259

CARAS

Res. Jr. of Agril. Sci. (Jul-Aug 2022)
13(4): 1255–1259

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

Changes in Biochemical Constituents and Enzymatic Activity of Moth Bean Seeds due to Infection of *Xanthomonas axonopodis* pv. *phaseoli*

Vishal Sharma[1], Kailash Agrawal*[1,3], Nanda Ram[1, 4] and Mukta Agrawal[2]

Received: 02 July 2022 | Revised accepted: 03 Aug 2022 | Published online: 15 Aug 2022

A B S T R A C T

Among pulses, moth bean seed contains a good amount of proteins, minerals (P, Fe, Ca, Mg, Zn), and vitamins (retinol, thiamine, riboflavin, niacin, pyridoxal phosphate, ascorbic acid, and folate). The present study was carried out on changes in biochemical constituents and enzymatic activity in moth bean seeds due to natural infection of *Xanthomonas axonopodis* pv. *phaseoli* (XAP). The infected seed samples have significantly low moisture, crude fiber, crude fat, crude protein, and total ash content while increased carbohydrate content as compared to healthy seed samples. Enzymatic activity (Cellulase, Peroxidase, and Polyphenol oxidase) was also significantly higher in infected seeds as compared to healthy seeds. Thus, the infection of XAP causes a loss in the nutritional quality of moth bean seeds.

***Key words*:** *Xanthomonas axonopodis* pv. *phaseoli*, Biochemical constituents, Enzymatic activity, Moth bean seeds

In the rural and arid places of Rajasthan (India), Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] is the main source of protein and calories. The Moth bean is a rich source of carbohydrates, protein, essential minerals, and vitamins and provides nutritional security to vegetarians in the arid region [1]. Raw and uncooked moth bean seeds provide energy similar to cereals. Moth bean is a good source of minerals namely calcium, iron and zinc, and vitamins which are beneficial for health [2-3]. Moth bean can be also used as green manure or forage in summer, helping to produce stocker cattle or as store food in the southern Great Plains [4]. Many bacterial and fungal species are responsible for various diseases in legume plants, but the most severe seed-borne bacterial disease is caused by *Xanthomonas axonopodis* pv. *phaseoli* (XAP), resulting in a decrease in the yield [5]. In hot areas, XAP is a dangerous threat, causing common bacterial blight, and responsible for maximum loss [6]. However, the primary association of *X. axonopodis* as a causal organism for causing disease has been observed in *Phaseolus vulgaris* (common bean) with some other bean crops viz. *Vigna aconitifolia* (moth bean), *V. radiata* (mung bean), *Phaseolus lunatus* (lima bean), and *Lablab purpureus* (lablab bean), which indicated the large host range of the pathogen [7-8].

Bacterial pathogens are responsible for the transmission of periodic as well as persistent diseases in plants [9]. *Xanthomonas* after entry into plants increase their number and disperse in all plant organs via the xylem, consuming nutrients from the cells of plants and resulting in death [10]. Seeds are also affected by a pathogenic infection resulting in the production of different proteolytic, lipolytic, and pectolytic enzymes in the infected seeds, and the content of protein-lipid and carbohydrate are affected. Peroxidase and polyphenol oxidase enzymes are also activated by the contact of host and parasite [11-12]. In the present study, changes in biochemical constituents and enzymatic activity of moth bean seeds due to infection of XAP have been investigated.

MATERIALS AND METHODS

To study the impact of infestation on biochemical constituents and enzymatic activity of moth bean seeds three seed samples of moth bean containing natural infection of *Xanthomonas axonopodis* pv. *phaseoli* (XAP) having a percent incidence of 71.25% ac no. M1215, 70.0% ac no. M1155, and 69.5% ac no. M1136 on semi-selective XCP_1 medium were selected and used in triplicates Healthy seed samples having ac. nos. M1109, M1175, and M1228 were used as control.

Analysis of biochemical constituents

* **Kailash Agrawal**
✉ agkailashindia@gmail.com

[1] Department of Botany, University of Rajasthan, Jaipur - 302 004, Rajasthan, India

[2] Department of Home Science, University of Rajasthan, Jaipur - 302 004, Rajasthan, India

[3] Department of Life Science, Vivekananda Global University, Jaipur - 303 012, Rajasthan, India

[4] Department of Botany, Shri Kalyan Rajkiya Kanya Mahavidyalaya, Sikar - 332 001, Rajasthan, India

Biochemical constituents of moth bean seeds were analyzed and calculated in percentage using standard methods of the Association of Official Analytical Chemists [13]. For the measurement of moisture and ash weight difference method was employed. For crude fat estimation, crude ether extract of moisture-free dry material was used. The solvent extraction method was used for the preparation of crude ether extract in the Soxhlet continuous extraction apparatus. Acid-alkali wash method was used for the calculation of the total fiber content of moisture-free and fat-free seed samples. The Kjeldahl method was used for the estimation of crude protein content. The carbohydrate content was calculated by the difference method [14].

Enzymatic activity

The change in activities of peroxidase, polyphenol oxidase, and cellulase enzymes was estimated by colorimetric methods [15]. Incubation of a few moth bean seeds using the standard blotter method was done for one week. Then incubated seeds (300 mg) were macerated in 0.1M concentrated (3 ml) phosphate buffer solution (pH 6.5). Centrifugation of the homogenous mixture was done for 15 min at 10000 rpm and 4°C. The supernatant was used to study the enzymatic assay [16]. When enzyme (0.2 ml) and O- dianisidine solution (0.1 ml) (1 mg/ml in methanol) was added to phosphate (0.1 M) buffer of pH 6.5 in a cuvette, the reaction started. The temperature of the mixture was maintained at 28-30ºC, then the cuvette containing the mixture was placed in a spectrophotometer (at wavelength 430nm). Then H_2O_2 (0.2 ml, 0.2 M) was added to the mixture. The time and initial absorbance A_0 were measured and recorded. Then values of absorbance were recorded again and again up to 3 min after every 30s duration. A graph was made for values of absorbance concerning time. The activity of enzyme peroxidase was represented in the form of OD (optical density) per unit time per mg seed weight.

For poly phenol oxidase enzyme activity, prepared the catechol solution by taking 0.01 catechol in 0.1 M PO_4^{2-} buffer, pH 6.0. 0.1 ml of enzyme extract was added to 3 ml catechol solution. Due to oxidation of catechol change in colour was observed and recorded again and again up to 5 min at 495 nm at a fixed interval of every 30s. A graph was made for values of absorbance w.r.t. time. The activity of enzyme polyphenol oxidase was represented in the form of an increase in OD (optical density) per unit time per mg seed weight.

For the estimation of enzyme activity of cellulase enzyme, 1 ml of enzyme extract was added to 0.5 ml of 0.1 M acetate buffer. Equilibrated this mixture at the temperature of 30ºC. 1.5 ml carboxymethyl cellulose was added to the above reaction mixture and then incubated at 30ºC for 2 hours. Then the reaction mixture was allowed to cool and absorbance was recorded (560 nm). A graph was made for values of cellulase activity of the unknown sample. The activity of enzymes was represented in the form of equivalent of n moles glucose, which was released in 1 h.

RESULTS AND DISCUSSION

In the present study, changes in moisture, fiber, fat, protein, ash, and carbohydrates due to infection of *X. axonopodis* pv. *phaseoli* were investigated in seeds of moth bean (Table 1).

Table 1 Effect on biochemical constituents of moth bean seeds due to natural infection of *X. axonopodis* pv. *phaseoli*

Sample accession No.		Biochemical constituents					
		Moisture (%)	Crude fiber (%)	Crude fat (%)	Crude protein (%)	Ash (%)	Total carbohydrate (%)
Check (Healthy seed samples)							
M1109	R_1	12.57	3.57	0.70	23.18	2.87	60.35
	R_2	12.62	3.35	0.75	23.22	2.79	60.69
	R_3	12.53	3.21	0.69	23.20	2.82	60.85
M1175	R_1	12.09	3.67	0.71	23.29	2.75	60.81
	R_2	12.15	3.85	0.68	23.34	2.72	60.63
	R_3	12.4	3.77	0.67	23.36	2.80	60.25
M1228	R_1	12.60	3.47	0.71	23.17	2.69	60.67
	R_2	12.65	3.49	0.72	23.27	2.74	60.50
	R_3	12.51	3.53	0.70	23.32	2.77	60.42
Mean		12.46	3.55	0.70	23.26	2.77	60.57
SD		0.21	0.19	0.02	0.07	0.07	0.21
Infected seed samples							
M1136	R_1	10.80	2.98	0.36	21.65	2.01	64.27
	R_2	10.95	3.10	0.33	21.75	1.99	64.45
	R_3	10.01	3.17	0.39	20.89	2.02	64.52
M1155	R_1	10.81	2.75	0.39	20.80	2.01	64.42
	R_2	10.90	2.69	0.31	21.05	1.98	64.19
	R_3	10.01	2.55	0.42	20.95	1.96	64.32
M1215	R_1	10.08	2.80	0.40	22.15	1.95	64.75
	R_2	10.12	2.69	0.35	21.23	2.02	64.71
	R_3	10.22	2.99	0.29	21.33	2.11	64.13
Mean		10.43	2.86	0.36	21.31	2.01	64.42
SD		0.42	0.21	0.04	0.45	0.05	0.22
Difference (2-1)		-2.02	-0.69	-0.34	-1.95	-0.77	3.84
SEd		0.15	0.09	0.02	0.15	0.02	0.09
t value		13.08	7.11	20.70	12.70	31.98	-38.56
p		*0.00	*0.00	*0.00	*0.00	*0.00	*0.00

SD – Standard deviation; SEd–Standard error of difference

*P is less than 0.01 therefore difference between test seed samples and infected seed samples is significant for all characters at 1%

Biochemical constituents

The mean moisture content was 12.57, 12.21, and 12.59% in the healthy seed samples (ac. no. M1109, M1175, and M1228) respectively while the moisture content of infected seed samples was 10.59, 10.57, and 10.14% of infected seed samples (ac. no. M1136, M1155, and M1215) respectively. The mean moisture content in healthy seed samples (check) was 12.46% whereas the mean moisture content of infected seed samples was lower to be 10.43% and significant at 1% (P≥0.01) (Table 1). Similarly lowering of moisture content was also reported in some other research regarding different bacterial pathogens viz. *Pseudomonas syringae* pv. *apii* [17] and *Xanthomonas axonopodis* pv. *phaseoli* [18] causing disease in ajwain and lentils respectively. Earlier it has been reported that swinging in the moisture content of seed detected in the pre-harvested state was proved as the main factor with regards to deterioration and it was involved in the reduction of seed germination of vigor [19-20]. The reason behind the sensitivity of seeds for variation in moisture content is directly connected to the composition of seed, especially for soybean, it is involved in two main processes viz. lipid peroxidation and protein degradation [21-23].

The crude fiber content was 3.08, 2.66, and 2.83% in infected seed samples (ac. no. M1136, M1155, and M1215) respectively whereas the crude fiber content values were 3.38, 3.76, and 3.50% of healthy seed samples (ac. no. M1109, M1175, and M1228) respectively. The mean crude fiber content of healthy seed samples (check) was 3.55% whereas in infected seed samples the value was 2.86%. The infected seed samples had lower crude fiber content (P≥0.01) when compared with a check (Table 1). Complex organic material hydrolysis could also be responsible for lowering the fiber content in the infected plants. Similar results were found when *Parthenium* spp. had an infection of *X. campestris* pv. *parthenii* [24].

The crude fat content was 0.36, 0.37, and 0.35 % in infected seed samples (with ac. no. M1136, M1155, and M1215) respectively whereas the crude fat contents of healthy seed samples were 0.71, 0.69, and 0.71% with ac. no. M1109, M1175, and M1228 respectively. The mean crude fat content of healthy seed samples (in check) was 0.70% whereas in infected seed samples the value was lower to be 0.36% (P≥0.01). Reduction of fat content in asymptomatic seeds of sunflower having an infection of *Pseudomonas syringae* pv. *syringe* has been reported [25].

The crude protein content was 21.43, 20.93, and 21.57% in infected seed samples (ac. no. M1136, M1155, and M1215) respectively whereas the crude protein content values were 23.2, 23.33, and 23.25% in 3 healthy seed samples (ac. no. M1109, M1175, and M1228) respectively. The mean crude protein content of infected seed samples (21.31%) was significantly (1% level) lower than healthy seed samples (23.26%) (Table 1).

The ash content of infected seed samples (ac. no. M1136, M1155, and M1215) was 2.01, 1.98, and 2.03%, respectively. In the case of healthy seed samples (ac. no. M1109, M1175, and M1228) the ash content was 2.83, 2.76, and 2.73% respectively. The mean ash content of infected seed samples (2.01%) was significantly (1% level) lower than healthy seed samples (in check) (2.77%) (Table 1). Similar results were found in soybean, pea, and bean seeds which had a natural infection of two fungi viz. *Fusarium moniliforme* and *Aspergillus parasiticus* [26].

The carbohydrate content was 64.41, 64.31, and 64.53% of infected seed samples (ac. no. M1136, M1155, and M1215 respectively) whereas the carbohydrate content values were 60.63, 60.56, and 60.53% in 3 healthy seed samples (ac. no. M1109, M1175, and M1228 respectively). The mean carbohydrate content of infected seed samples (64.42%) was significantly higher than healthy seed samples (in check) (60.57%) (Table 1).

Lowering of protein, crude fiber, and fat was observed in seeds of cowpea having natural infection of *Colletotrichum destructivum* and pathogen consume the nutrition as metabolic carbon for obtaining energy and amino acids for increasing protoplasm and nitrogen are necessary elements for the reproduction and growth of these fungi [27]. The infected cowpea seeds showed a lowering in phosphorus, calcium, carbohydrate, protein, crude fiber, ash, and lipid and an elevation in the sodium, iron, potassium, magnesium, zinc, and phosphorus accumulation [28].

Enzymatic activity

The mean peroxidase activity in healthy samples seed (in check) was 0.42 ΔOD/min/mg seeds whereas in infected seed samples the value was 1.33 ΔOD/min/mg seeds. So, the difference of 0.90 ΔOD/min/mg seeds in peroxidase activity was found statistically significant at 1% (P≥0.01). As compared to the check all the infected seed samples had higher peroxidase activity values (Table 2), it has been found that peroxidase enzymes were the reason for the formation of the high amount of hydrogen peroxide and superoxide anions (free radicals) in infected tissues of the plants [29]. In diseased seed samples, the mean peroxidase value was found to be significantly higher than in asymptomatic seed samples. Similar results were also found in diseased plants of cotton infected with *X. campestris* pv. *malvacearum* with a decrease in peroxidase level [30].

The mean polyphenol oxidase activity in healthy seed samples (in check) (0.272 ΔOD/min/mg seeds) was significantly lower than the mean polyphenol oxidase activity of infected seeds. It has been proved that polyphenol oxidase is a nuclear coding protein, containing copper in its structure, and catalyses the hydroxylation of phenols into quinines [31]. In diseased seeds of ajwain infected with *Pseudomonas syringae* pv. *apii*, the activity of enzyme polyphenol oxidase was found higher than the mean value of healthy samples of seeds. These findings correspond to the work of Chitoor [32] and Shivalingaiah and Umesha [33]. Due to the effect of the pathogen on enzymatic activities of peroxidase and polyphenol oxidase, the metabolic activity of the plant was also changed due to the mutual interaction of pathogen and host [34-35]. It was also noted that when plants were attacked by their compatible pathogen, the amount of ROS (reactive oxygen species) viz. hydroperoxyl radicals, superoxide, and hydrogen peroxide increased significantly [36].

The mean cellulase activity was 0.583, 0.577, and 0.568 mM glucose released/h in infected seed samples having ac. no. M1136, M1155, and M1215 respectively were 0.576 mM glucose released/h whereas the mean cellulase activity in healthy seed samples (samples ac. no. M1109, M1175, and M1228 respectively) was 0.225 mM glucose released/h. So, the difference in cellulase activity was found significant at 1% (P≥0.01).

Changes in cellulolytic and pectinolytic enzyme activity during pathogenesis have been reported by various workers [37-38]. Due to infection by various pathogens, a significant increase in the activities of enzymes viz. peroxidase and polyphenol oxidase was reported [39-41]. Similar work was also done for the detection of *P. syringae* pv. *apii* on enzymatic activity and profiling of nutritional value and essential oil of ajwain seeds [17].

Table 2 Changes in enzymatic activity of moth bean seeds infected with *Xanthomonas axonopodis* pv. *phaseoli*

Sample accession No		Enzymatic activity		
		Peroxidase (ΔOD/min/mg seeds)	Polyphenol oxidase (ΔOD/min/mg seeds)	Cellulase (m Mole glucose released/h)
Check (Healthy seed samples)				
M1109	R_1	0.416	0.273	0.219
	R_2	0.407	0.261	0.223
	R_3	0.422	0.269	0.231
M1175	R_1	0.425	0.276	0.217
	R_2	0.427	0.266	0.227
	R_3	0.419	0.271	0.229
M1215	R_1	0.428	0.274	0.225
	R_2	0.424	0.278	0.228
	R_3	0.431	0.281	0.222
Mean		0.422	0.272	0.225
SD		0.007	0	0.004
Infected seed samples				
M1136	R_1	1.329	1.15	0.575
	R_2	1.378	1.176	0.581
	R_3	1.393	1.181	0.593
M1155	R_1	1.292	1.167	0.569
	R_2	1.351	1.148	0.577
	R_3	1.286	1.169	0.586
M1215	R_1	1.316	1.144	0.564
	R_2	1.289	1.159	0.561
	R_3	1.304	1.177	0.58
Mean		1.326	1.163	0.576
SD		0.04	0.01	0.01
Difference (2-1)		0.904	0.891	0.352
SEd		0.013	0.005	0.003
t value		-67.41	-177.52	-93.17
p		*0.00	*0.00	*0.00

SD – Standard deviation; SEd–Standard error of difference

*P is less than 0.01 therefore difference between test seed samples and infected seed samples is significant for all characters at 1%

CONCLUSION

Natural infection of seed samples of moth bean with *X. axonopodis* pv. *phaseoli* caused a significant lowering of moisture, crude fiber, crude fat, crude protein, and ash content. The enzymatic activity viz. peroxidase, polyphenol oxidase, and cellulase in naturally infected seeds of moth bean infected with *X. axonopodis* pv. *phaseoli* were significantly higher than healthy (check) seed samples. Thus, infection of *X. axonopodis* pv. *phaseoli* cause a loss in the nutritional quality of moth bean seeds.

Acknowledgment

University Grants Commission (UGC) New Delhi is duly acknowledged for Junior and Senior Research Fellowship (JRF & SRF) to Vishal Sharma and the Head, Department of Botany, University of Rajasthan, Jaipur is acknowledged for providing research facilities.

LITERATURE CITED

1. Kumar D. 2002. Production Technology for Moth bean in India. *AICRP on Arid Legume.* CAZRI, Jodhpur, Rajasthan. pp 41.
2. Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J. 2000. Structure of genetic diversity among common bean land races of Middle American origin based on correspondence analysis of RAPD. *Crop Science* 40: 264-273.
3. Parul B. 2014. Anti-nutritional factors in foods and their effects. *Journal of Academia and Industrial Research* 3(6): 285-290.
4. Baath GS, Northup BK, Gowda PH, Turner KE, Rocateli AC. 2018. Moth bean: A potential summer crop for the Southern Great Plains. *American Journal of Plant Sciences* 9: 1391-1402.
5. Sallam NMA. 2011. Biological control of common blight of bean (*Phaseolus vulgaris*) caused by *Xanthomonas axonopodis* pv. *phaseoli* by using the bacterium *Rahnella aquatilis*. *Archives of Phytopathology and Plant Protection* 44(20): 1966-1975.
6. Silue S, Jacquemin J, Baudoin J. 2010. Utilisation des mutations induites pour l'etude de l'embryogenese chez le haricot *Phaseolus vulgaris* L. et deux plantes modeles, *Arabidopsis thaliana* (L.) Heynh. et *Zea mays* L. *Biotechnology, Agronomy and Society and Environment* 15(1): 195–205.
7. Saettler AW. 1991. *Common Bacterial Blight. In*: (Eds) Hall R. Compendium of Bean Diseases. St. Paul, MN, USA. pp 29-30.
8. Schwartz HF, Steadman JR, Hall R, Forster RL. 2005. Compendium of Bean Diseases. St. Paul, MN, *American Phytopathological Society*. pp 109.
9. Ogolla FO, Neema DB. 2019. Cultural, morphological and biochemical identification of *Xanthomonas* spp. the causative agent of bacteria leaf spot in tomatoes in Wanguru, Mwea, Kirinyaga County, Kenya. *International Journal of Research and Innovation in Applied Science* 4(4): 44-49.
10. Narvekar S, Thilakan A, Bhangale M, Yeri A, Pawar V, Kasar N, Shukla P, Trivedi M 2019. Isolation, identification and *in vitro* antibiotics sensitivity pattern of local isolates of *Xanthomonas* followed by production of xanthan gum using agro-industrial waste. *Journal of Emerging Technologies and Innovative Research* 6(5): 181-188.

11. Tripathi RK, Verma MN. 1975. Phenolic compounds and polyphenol oxidase activity in relation to resistance in potatoes against bacterial soft rot. *Indian Journal of Experiment Biology* 13: 414-416.
12. Hammerschmidt R, Nuckles EM, Kuć J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology* 20(1): 73-82.
13. AOAC. 1955. *Official Methods of Analysis*. (Eds) Hornitz W. AOAC. Washington D.C. pp. 1995.
14. Raghuramulu N, Madhavan NK, Kalyansundaram S. 2003. A manual of laboratory techniques NIN Press, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. pp 424.
15. Malik CP, Singh MB. 1980. *Plant Enzymology and Histo-Enzymology*: A text manual. Kalyani Publications, New Delhi/Ludhiana. pp 434.
16. Mahatma MK, Bhatnagar RP, Rawal P. 2008. Changes in enzymes and proline levels in leaves of downy mildew resistant and susceptible pearl millet genotypes. *Journal of Mycology and Plant Pathology* 38(2): 277-281.
17. Ram N, Agrawal K, Sharma V, Khorwal S, Agrawal M. 2021. Effect of *Pseudomonas syringae* pv. *apii* on nutritional value, essential oil profiling and enzymatic activity of ajwain (*Trachyspermum ammi* L.) seeds. *Research Journal of Agricultural Sciences* 12(4): 1419-1424.
18. Kulshrestha S. 2015. Seed-borne bacterial diseases of lentil (*Lens culinaris* medik.), their phytopathological effects and disease management. *Ph. D. Thesis*, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. pp 182.
19. Malik CP. 2013. Seed deterioration: a review. *International Journal of Life Science and Pharma Research* 2: 374-385
20. Marcos-Filho J. 2016. *Seed Physiology of Cultivated Plant*. ABRATES, Londrina, PR, Brazil.
21. Xin X, Tian Q, Yin G, Chen X, Zhang J, Ng S, Lu X. 2014. Reduced mitochondrial and ascorbate-glutathione activity after artificial aging in soybean seed. *Journal of Plant Physiology* 171: 140-147.
22. Min CW, Kim YJ, Gupta R, Kim SW, Han WY, Ko JM, Kang HW, Yoon WB, Choung MG, Kim YC, Kim ST. 2016. High-throughput proteome analysis reveals changes of primary metabolism and energy production under artificial aging treatment in *Glycine max* seeds. *Applied Biological Chemistry* 59: 841-853.
23. Min CW, Lee SH, Cheon YE, Han WY, Ko JM, Kang HW, Kim YC, Agrawal GK, Rakwal R, Gupta R, Kim ST. 2017. In-depth proteomic analysis of *Glycine max* seeds during controlled deterioration treatment reveals a shift in seed metabolism. *Journal of Proteomics* 169: 125-135.
24. Sain SK, Gour HN. 2008. Pathological, physiological and biochemical characterisation of *Xanthomonas axonopodis* pv. *parthenii* incident of leaf blight in *Parthenium hysterophorus. Journal of Mycology and Plant Pathology* 38(3): 466-477.
25. Godika S, Agarwal K, Singh T. 2000. Histopathological and biochemical changes in *Pseudomonas syringae. Indian Phytopathology* 50: 1131-1132.
26. Embaby EM, Mohamed R, Mosaad A, Abdel W, Hassan O, Asmaa MM. 2013. Occurrence of toxigenic fungi and mycotoxins in some legume seeds. *International Journal of Agricultural Technology* 9(1): 151-164.
27. Sangeetha CA, Rawal RD. 2008. Nutritional studies of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. the incitant of mango anthracnose. *World Journal of Agricultural Science* 4(6): 717-720.
28. Amadioha AC, Nwazuo ED. 2019. Biochemical Composition of Seed and Husk of Cowpea (*Vigna unguiculata* (L.) Walp.) Infected by *Colletotrichum destructivum* O'Gara in Storage. *Annual Research and Review in Biology* 31(1): 1-7.
29. Martinez C, Montillet JL, Bresson E, Agnel JP, Dai GH, Daniel JF, Geiger JP, Nicole M. 1998. Apoplastic peroxidase generates superoxide anions in cells of cotton cotyledons undergoing the hypersensitive reaction to *Xanthomonas campestris* pv. *malvacearum* race 18. *Molecular Plant Microbe Interaction* 11: 1038-1047.
30. Delannoy E, Jalloul A, Assigbetse K, Marmey P, Geiger JP, Lherminier J, Daniel JF, Martinez C, Nicole M. 2003. The activity of class III peroxidases in defense of cotton to bacterial blight. *Molecular Plant Microbe Interaction* 16(11): 1030-1038.
31. Mishra BB, Gautam S. 2016. Polyphenol oxidases: Biochemical and molecular characterization, distribution, role and its control. *Enzyme Engineering* 5(1): 1-9.
32. Chittoor JM, Leach JE, White FF. 1997. Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. *oryzae*. *Molecular Plant Microbe Interaction* 10: 861-871.
33. Shivalingaiah, Umesha S. 2016. Study of the involvement of peroxidase and polyphenol oxidase in imparting resistance to bacterial blight disease in *Oryza sativa* varieties. *European Journal of Biotechnology and Bioscience* 4(7): 5-10.
34. Salari M, Panjekeh N, Nasirpoor Z, Abkhoo J. 2012. Changes in total phenol, total protein and peroxidase activities in melon (*Cucumis melo* L.) cultivars inoculated with *Rhizoctonia solani*. *Afr. Jr. of Microbiology Research* 6(37): 6629-6634.
35. Ngadze E, Icishahayo D, Coutinho TA, Van der Waals JE. 2012. Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. *Plant Disease* 96: 186-192.
36. Do HM, Hong JK, Jung HW, Kim SH, Ham JH, Hwang BK. 2003. Expression of peroxidase-like genes, H_2O_2 production, and peroxidase activity during the hypersensitive response to *Xanthomonas campestris* pv. *vesicatoria* in *Capsicum annuum*. *Molecular Plant-Microbe Interactions* 16(3): 196-205.
37. Prasad BK, Shanker U, Narayan N, Kishor A, Dayal S. 1988. Alteration in the enzyme activities of seeds of finger millet due to *Aspergillus flavus*. *Indian Phytopathology* 41: 578-580.
38. Reddy PB, Reddy SN. 1988. Role of oxidases and cell degrading enzymes in the fruit rot of *Coccinia* caused by *Rhizoctonia solani*. *Indian Phytopathology* 41: 138-141.
39. Addy SK, Goodman RN. 1972. Polyphenol oxidase and peroxidase activity in apple leaves inoculated with a virulent or avirulent strain of *Erwinia amylovora*. *Indian Phytopathology* 25: 575-579.
40. Dankevych L, Leonova N, Dragovoz I, Patyka V, Kalinichenko A, Wlodarczyk P, Wlodarczyk B. 2019. The synthesis of plant growth stimulators by phytopathogenic bacteria as factor of pathogenicity. *Appl. Ecol. and Environ. Res.* 16: 1581-1593.
41. Kolomiiets, YV, Grygoryuk IP. Butsenko LM, Kalinichenko AV. 2019. Biotechnological control methods against phytopathogenic bacteria in tomatoes. *Applied Ecology and Environmental Research* 17: 3215-3230.