African Journal of Rural Development, Vol. 3 (3): July- September 2018: pp. 859-868. ISSN 2415-2838

Biochemicals associated with *Callosobruchus chinensis* resistance in soybean

U.M.MSISKA[1, 2], B.W. MIESHO,[1] M.G. HAILAY,[1], S. KYAMANYWA,[1] P. RUBAHAIYO,[1] T. ODONG,[1] P. TUKAMUHABWA,[1] E. NUWAMANYA, [1] and D.L. NABIRYE,[1]

[1]Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda

[2]Department of Crop Development, Mzuzu ADD, P. O. Box 131, Mzuzu, Malawi

Corresponding author: ulemsiska@gmail.com

ABSTRACT

Soybean (*Glycine max* L.) is a very important crop worldwide because of its high protein and oil content. However, soybean suffers damage from bruchid (*Callosobruchus chinensis*) during storage. Understanding of factor(s) contributing to bruchid resistance is useful for development of soybean cultivars with genetic resistance to bruchid damage. Biochemicals associated with resistance to *Callosobruchus chinensi*s were investigated on eight soybean genotypes with varying levels of resistance. Significant differences (P<0.05) were observed amongst genotypes with regards to antinutritional factors particularly total antioxidants (TAOX), tannins, peroxidase (POD), and flavonoids. There were no significant differences among genotypes with regards to nutritional factors particularly proteins, starch, lipid peroxidation, and reducing sugars. A resistant genotype AVRDC G8527 had the highest concentration of total antioxidants (1.98 AU min^{-1} mg^{-1}) and tannins (1.85mg TAE $100g^{-1}$) followed by Maksoy 3N while the least was in a very susceptible genotype AGS 292 (TAOX=0.39 AU min^{-1} mg^{-1}, Tannin=0.296 mg TAE $100g^{-1}$). AGS 292 had the highest concentration of flavonoids (31.22 mg QE 100g-1) while AVRDC G8527 had the least (5.13 mg QE $100g^{-1}$). Peroxidase activity was highest in AVRDC G89 (0.27AU min^{-1} mg^{-1}) while AGS 292 had the least (0.07AU min^{-1} mg^{-1}). From the correlation analysis, there was a significant strong negative relationship between flavonoids and tannin (r=-0.73**) and TAOX (r=- 0.71**) but a positive non-significant relationship with phenolic (r=0.22) and alkaloids (r=0.37) was recorded. Peroxidase activity had a significant relationship with median development period (r=0.69*) indicating that increased peroxidase activity resulted into increased seed resistance through prolonged insect development period. There was a strong relationship between tannins and total antioxidants (r=0.99**). These results indicate that secondary metabolites, especially peroxidase, tannin, and TAOX were the biochemicals associated with higher resistance to *C. chinensis* in soybean while flavonoids were associated with higher susceptibility.

Key words: Alkaloids, antinutritional-factors, bruchids, lipids, phenolics, soybean

RÉSUMÉ

Le soja (*Glycine max* L.) est une culture très importante dans le monde entier en raison de sa teneur élevée en protéines et en huile. Toutefois, le soja est endommagé par le Callosobruchus chinensis pendant le stockage. La compréhension des facteurs contribuant à la résistance au Callosobruchus chinensis est utile pour le développement de cultivars de

Cite as: Msiska, U. M., Miesho, B. W., Hailay, M. G., Kyamanywa, S., Rubahaiyo, P., Odong, T., Tukamuhabwa, P., Nuwamanya, E. and Nabirye, D. L. 2018. Biochemicals associated with Callosobruchus chinensis resistance in soybean. *African Journal of Rural Development* 3 (3): 859-868.

Received: 13 January 2018
Accepted: 15 May 2018
Published: 30 September 2018

soja présentant une résistance génétique. Des produits biochimiques associés à la résistance au Callosobruchus chinensis ont été étudiés sur huit génotypes de soja présentant des niveaux de résistance variables. Des différences significatives (P<0.05) ont été observées entre les génotypes en ce qui concerne les facteurs antinutritionnels, en particulier les antioxydants totaux (TAOX), les tanins, la peroxydase (POD) et les flavonoïdes. Il n'y avait pas de différences significatives entre les génotypes en ce qui concerne les facteurs nutritionnels, en particulier les protéines, l'amidon, la peroxydation lipidique et les sucres réducteurs. Un génotype résistant AVRDC G8527 présentait la concentration la plus élevée d'antioxydants totaux (1,98 AU min-1 mg-1) et de tanins (1,85mg TAE 100g-1), suivi de Maksoy 3N alors que le moins résistant était dans un génotype très sensible AGS 292 (TAOX=0,39 UA min-1 mg-1, Tannin=0,296 mg TAE 100g-1). L'AGS 292 présentait la concentration la plus élevée de flavonoïdes (31,22 mg QE 100g-1) tandis que l'AVRDC G8527 présentait la concentration la plus faible (5,13 mg QE 100g-1). L'activité de peroxydase était la plus élevée dans l'AVRDC G89 (0,27AU min-1 mg-1) tandis que l'AGS 292 présentait la plus faible activité (0,07AU min-1 mg-1). D'après l'analyse de corrélation, il y avait une forte relation négative significative entre les flavonoïdes et les tanins (r=-0,73**) et le TAOX (r=-0,71**), mais une relation positive non significative avec le phénol (r=0,22) et les alcaloïdes (r=0,37) a été enregistrée. L'activité de la peroxydase avait une relation significative avec la période de développement médiane (r=0,69*), ce qui indique qu'une activité accrue de la peroxydase a entraîné une résistance accrue des semences pendant une période prolongée de développement des insectes. Il y avait une forte relation entre les tanins et les antioxydants totaux (r=0,99**). Ces résultats indiquent que les métabolites secondaires, en particulier la peroxydase, le tannin et le TAOX, étaient les substances biochimiques associées à une plus grande résistance au C. chinensis dans le soja, tandis que les flavonoïdes étaient associés à une plus grande sensibilité.

Mots clés: Alcaloïdes, facteurs antinutritionnels, bruchides, lipides, phénoliques, soja

INTRODUCTION

Soybean (*Glycine max* L.) is a very important crop worldwide because of its high protein and oil content. It is thus a major component of infant formulae and animal feeds. However, soybean suffers serious damage from bruchid (*Callosobruchus chinensis*) during storage. Understanding of factor (s) and mechanisms contributing to bruchid resistance is useful for development of soybean cultivars with genetic resistance to bruchid damage, Plate 1. Resistance to pests involves morphological barriers and phytochemicals. In legumes there are two levels of biochemical based resistance observed; general resistance which prevents infestation by a wide range of pests, and specific resistance targeted at specific pest species, in particular those able to counteract the general resistance chemicals (Minney 1990). Usually biochemicals involved in resistance to pests are products of secondary metabolism. Secondary metabolites are organic compounds which are not involved in primary metabolism of the cell (Manisha, 2017). Secondary compounds accumulate in the seed and are considered to have a role specific to the seed since they often disappear soon after germination (Minney, 1990). There are probably 400,000 secondary compounds that play a defensive role in plants (Swain, 1977).

It was hypothesized by other researchers that metabolites content of soybean might vary in soybean genotypes and influence resistance to pests (Msiska *et al.,* 2018). The amount and kinds of these biochemicals in soybean genotypes with differences in susceptibility to *Callosobruchus chinensis* and their relationships to bruchid resistance are not well documented. Identification of biochemicals associated with bruchid resistance in soybean would facilitate selection of desired parents and cross combinations that enhance resistance to bruchids. Therefore, a study was carried out with the objective of identifying biochemicals that are associated with *C. chinensis* resistance in soybean. In this study it was hypothesized that (i) soybean contains more than one type of metabolite that are responsible for and associated with bruchid resistance; and (ii) soybean genotypes contain varying amounts of resistance metabolites.

MATERIALS AND METHODS

Biochemicals associated with resistance to *Callosobruchus chinensis* were investigated on eight soybean genotypes with varying levels of resistance to the bruchid. This work was done in the Nutritional and Bioanalytical laboratory based at the National Crops Resources Research Institute (NacRRI) in Uganda. Methods used in testing the various metabolites were adopted from different studies as presented in Table 1.

Table 1. Metabolites tested and Methods used in the study

ID	Metabolite	Method
1	Protein	Nuwamanya *et al.,* 2014
2	Starch	Parthiban *et al.,* 2012
3	Reducing Sugars	Nuwamanya *et al.,* 2014
4	Phenolics	Wong *et al.,* 2009
5	Flavonoids	Kale *et al.,* 2010
6	Total Antioxidants	Ahmed *et al.*, 2015
7	Alkaloids	Vijay and Rajendra, 2014
8	Lipid peroxidation	Health and Packer, 1966
9	Phytic acid	Yahaya *et al.,* 2013
10	Peroxidase	Shannon *et al.,* 1966
11	Tannins	Mrudula and Prabhu, 2014

Plate 1. Could this damage variability be associated with biochemical content?

RESULTS AND DISCUSSION

Analysis of variance showed significant ($P<0.05$) genotype effects for tannins, flavonoids, total anti-oxidants, phenolics, peroxidases and reducing sugars (Table 2). Genotypes did not significantly influence concentrations of alkaloids, lipid peroxidation, phytic acid, proteins and starch.

Results on the quantitative estimation of the studied metabolites are presented in Table 3. Quantities of anti-nutritional factors in seed particularly, tannins, total antioxidant and peroxidases were positively related to resistance to *C. chinensis*. These are highly thought to be responsible for resistance to *C. chinensis* in soybean (War *et al.,* 2012). The results also indicated that the compounds found in seeds acted either additively or synergistically against bruchids. They acted either directly on bruchid through antibiosis or induced non-preference in insects feeding on the seeds (War *et al.,* 2017). The secondary metabolites served to reduce or destroy palatability of soybean. Sharma and Thakur (2014) reported that anti nutritional and not nutritional factors were responsible for resistance in legumes. A similar finding has come out of this study.

Flavonoids concentration was highest in susceptible genotypes than in the resistant genotypes ($P<0.001$) indicating positive impact on bruchid development. The most susceptible genotype, AGS 292 had the highest concentration of flavonoids (31.22 mg QE per 100g) followed by Maksoy 2N (22.14 mg QE/100g) while AVRDC G 8527 had the least concentration of flavonoids (5.13mg QE/100g). Findings in this study showed that flavonoids were associated with increased susceptibility. This finding is in agreement with the report by Lattanzio *et al.* (2006) who reported that most plants contain an array of flavonoids which phytophagous insects usually differentiate. Lattanzio *et al.* (2006) went further to explain that some flavonoids are feeding and oviposition stimulants to insects implying that genotypes with high concentrations of such flavonoids will be susceptible to insect pest attack. Alkaline midgut pH, surfactants, and the peritrophic membrane all may help these species tolerate flavonoid concentrations in the diet. Furthermore, in comparison to many other secondary metabolites, flavonoids are apparently not very toxic to and have a low physiological activity in most insects (Lattanzio *et al.,* 2008.).

The highest phenol concentration was recorded in a resistant genotype AVRDC G89 (1051 mg of GAE per 100g) followed by AGS 292 (554.4 mg GAE per 100g) while the least concentration was recorded in Maksoy 1N (139.1 mg GAE per 100g). Significant differences observed in phenols among genotypes did not show any defined pattern between the susceptible and resistant genotypes suggesting that it might not have been responsible for susceptibility of genotypes to bruchids. Mahatma *et al.* (2011) observed similar results where genotypes reported increased content of phenolics but without conferring any resistance. This could be attributed to the fact reported by Wu *et al.* (2015) that plant phenolics were not toxic to insects unless prophenoloxidase genes are lost or the levels of phenolics exceed the catalytic activity of the gut prophenoloxidases. Prophenoloxidases which are produced in the foreguts detoxify phenols in the midgut of insects.

Tannin concentration in the genotypes ranged from 0.296 to 1.845 mg of tannic acid equivalents per 100g sample. Genotype AGS 292 had the lowest tannin concentration while AVRDC G8527 had the highest. Tannins are the most abundant secondary metabolites made by plants. The significant differences in tannin concentration amongst resistant and susceptible genotypes implied that tannin played a role in soybean resistance to *Callosobruchus chinensis*. Tannins are generally considered to be deleterious to phytophagous insects. Tannins may affect the growth of insects in three main ways: they have an astringent taste, which affects

Table 2. Results of one-way analysis of variance for differences in seed biochemical traits among the studied soybean

SOV	df	TAOX	Tannins	PA	Phenolics	LP	Flavonoids	Alkaloids	Protein	Starch
Blocks 2	0.986	0.237	10.078	0.003	0.191	0.005	0.008	0.053	0.386	
Genotypes	7	1.039	0.266	2.019	0.003	0.112	0.045	0.008	0.003	0.142
Residual 14	0.299	0.076	1.150	0.001	0.111	0.005	0.006	0.012	0.056	
P-Value	0.023	0.021	0.175	0.043	0.462	<0.001	0.369	0.952	0.065	

Sov= source of variation, df= degrees of freedom, TAOX= Total antioxidants, PA= Phytic acid, LP= Lipid peroxidation

Table 3. Estimates of seed biochemical constituents in resistant and susceptible soybean genotypes

Genotype	Status	Tannins	TAOX	Flavonoids	Alkaloids	Phenolics	LP	Phytic A	Protein	RS	Starch
		mgTAE /100g	AUmin^{-1} mg^{-1}	mgQE/100g	AUmin^{-1}mg^{-1}	mg GAE /100g	nmol MDA/g	mg/100g	mg/100g	mg/100g	mg/100g
G8527	R	1.845	1.978	5.13	0.17	148.5	0.01	10.15	33180.78	0.006	48.89
G89	R	1.21	1.324	5.88	0.19	1051.1	1.16	9.23	47069.67	0.006	50.13
Mak 1N	MR	1.013	1.176	6.11	0.16	139.1	0.01	10.04	39943.08	0.006	38.84
SL9.2	MR	0.968	1.112	10.91	0.25	249.7	0.15	10.96	40785.52	0.006	49.01
SL13.2A	MR	0.441	0.565	8.56	0.26	367.6	0.06	11.19	28900.27	0.006	64.13
Mak 3N	S	1.651	1.778	8.37	0.16	368.6	0.51	10.61	48367.49	0.007	27.58
Mak 2N	S	0.394	0.509	22.14	0.14	572.1	0.02	9.58	43221.77	0.005	38.56
AGS 292	S	0.296	0.391	31.22	0.27	554.4	0.02	8.88	35913.02	0.007	18.69
P-Value	0.021	0.023	<.001	0.369	0.043	0.462	0.175	0.952	0.035	0.065	
LSD	0.937	0.957	9.209	0.143	532.985	0.583	1.878	3859	0.56	0.00117	27.165

Mak 1N= Maksoy 1N, Mak3N= Maksoy 3N, Mak2N= Maksoy 2N, SL9.2= S-Line 9.2, SL13.2A= S-Line 13.2A, R= Resistant, MR=Moderate resistant, S=Susceptible, TAOX= Total antioxidants, TAE= tannic acid equivalent, QE =Quercetin equivalent, GAE= Gallic Acid equivalents, AU= Absorbance Units LP= Lipid peroxidation, PA= Phytic acid, RS= Reducing sugars, MDA= Malondialdehyde palatability, and decrease feed consumption, they form complexes of reduced digestibility with proteins and they act as enzyme inactivators (Winkel *et al.,* 1998). Barbehenn and Constabel (2011) reported that tannin toxicity in insects is thought to result from the production of high levels of reactive oxygen species which react with high pH guts, forming semiquinone radicals and quinones. When developing, larvae fed on the tanniferous soybean, tannins permeated the peritrophic envelopes thereby producing fatal lesions in insects' midgut which subsequently led to reduced growth of insects in the resistant soybean genotypes (Barbehenn and Constabel, 2011).

Total antioxidants (TAOX) concentration was highest in resistant genotypes and lowest in susceptible genotypes ($P<0.023$) indicating that TAOX negatively impacted bruchid growth and development. AVRDC G8527 had the highest total antioxidants concentration (1.98 AU $min^{-1}mg^{-1}$) followed by Maksoy 3N (1.78 AU $min^{-1}mg^{-1}$) while susceptible AGS 292 had the least concentration (0.39 AU $min^{-1}mg^{-1}$). Presence of antioxidants indicated the capacity of a genotype to cause vital damage to organs in insects which is a firm defense mechanism related to bruchids (Kolawole and Kolawole 2014).

Alkaloid concentration in soybean seeds ranged from 0.14 - 0.27 mg of AE per g of extract. The highest content was measured in AGS 292 and the lowest in Maksoy 2N. Nevertheless there was no significant genotype effect on alkaloid concentration, indicating that alkaloids might not be playing part in soybean resistance to *C. chinensis.*

The highest phytic acid was in S-Line 13.2A (11.19mg/100g) and lowest was in AGS 292 with 8.88mg per 100g. All genotypes contained phytic acids (PA) but with no significant differences suggesting that the resistance to *C. chinensis* in soybean was not due to PA. Dhole and Reddy (2016) reported that higher concentrations of PA were required for the resistant reaction in mungbean. Dhole and Reddy (2016) further indicated that even though the resistant gene may be present in a plant, reduced concentration of PA decreases tolerance to biotic stress. Under this reasoning therefore it was speculated that PA concentration in soybean was not high enough to affect bruchid's metabolic activities, growth and development.

The study established that there were no significant differences in proteins, lipid peroxidation and starch amongst genotypes implying that these nutritional factors were not associated with resistance to *C. chinensis* in soybean. Even in reducing sugars where genotypes showed significant differences there was no trend observed between the resistant and susceptible genotypes. This finding is of significant importance in soybean breeding programs for resistance to *C. chinensis* because it implies that nutritional factors of soybean will not be affected with breeding towards more bruchid resistance. These findings are in agreement with those of Sharma and Thakur (2014) who reported that nutritional factors were not responsible for resistance to bruchids in chickpea, cowpea and soybean.

The results on peroxidase activity for the studied genotypes over a period of 150 seconds showed significant variations amongst genotypes ($P<0.001$), Table 4. However, peroxidase activity was not statistically affected by time ($P=0.998$) and the interaction between time and genotypes ($P=1.00$). Resistant genotype G89 had the highest peroxidase activity (0.27 AU min^{-1} mg^{-1}) followed by genotype S-Line 13.2A (0.18 AU min^{-1} mg^{-1}) while the least activity was observed on a susceptible genotype AGS 292 (0.07 AU min^{-1} mg^{-1}) indicating that peroxidase negatively impacts *C. chinensis'* growth and development in soybean. Peroxidases deter the feeding of insects and produce toxins that reduce plant digestibility, which in turn leads to nutrient deficiency in insects with drastic effects on their growth and development (War *et al.,* 2012). Furthermore peroxidases impair nutrition through forming electrophiles which oxidize mono-or dihydroxyphenols thereby directly causing toxicity in the guts of the insects (Zhu-Salzman *et al.,* 2008). Peroxidase in the presence of hydrogen peroxide catalyzes autoxidation of tannin compounds. Oxidized tannins do react with proteins and decrease their nutritional quality (Barbehenn and Constabel, 2011).

Correlation analysis between the susceptibility and biochemical parameters indicated that anti-nutritional factors such as tannins, total antioxidants and peroxidase were associated with seed resistance in soybean. Therefore it can be deduced that soybean genotypes with high tannin and total antioxidants would have lower number of adult insect emergence, longer median development periods, smaller DSI values, lower seed weight percent loss, delayed growth index and consequently would be resistant to *C. chinensis* attacks. In contrast, correlation analysis revealed that high flavonoid concentration would favour high numbers of adult emergence, seed weight loss, shorter development periods and consequently higher DSI implying more susceptibility to *C. chinensis*. This finding therefore means that when breeding for bruchid resistance flavonoids should be selected against.

The negative relationship between peroxidase and DSI, adult emergence, weight loss and insect growth index indicate that peroxidase contributed positively to seed resistance to bruchids. This finding is in agreement with that of Khan *et al.* (2003) who reported that resistance-related enzymes such as chitinase, β-1,3-glucanase, and peroxidase are also involved in the bruchid resistance. Lattanzio *et al.* (2006) reported that the effectiveness of phenolics as resistance factors to insect feeding is enhanced by oxidation to polymers, which reduce digestibility, palatability and nutritional value. Thus high levels of polyphenol oxidases and peroxidases, the major phenolic oxidising enzymes of plants, can be correlated with plant resistance mechanisms against insects. The non-significant relationship between peroxidase and seed weight parameters indicate that resistance to *C. chinensis* in soybean is independent of seed size. This finding is in agreement with that of Sharma and Thakur (2014b) who reported that seed size had no influence on susceptibility parameters of chickpea, soybean and cowpea.

Significant positive correlation between peroxidase activity and median development period implied that peroxidase activity contributed to slow development of *C. chinensis.* Slow development means less number of generations per year. As such, the result consequently suggest that genotypes with higher peroxidase activity would be more resistant to *C. chinensis*. Therefore peroxidase

Table 4. Peroxidase Activity for eight genotypes over 150 Seconds

SOV	d.f.	Mean Squares	F pr.
Blocks	2	0.060317	
Genotype	7	0.079175	<0.001
Time	5	0.000492	0.998
Genotype X Time	35	0.000016	1.00
Residual	94	0.008848	
Total	143	1.509642	

is amongst the biochemicals associated with *C. chinensis* resistance in soybeans. Results of this study are in agreement with that of Sharma and Thakur (2014a), that peroxidase affected insect metabolic activities and inhibited growth of larvae in soybean hence the longer development periods. From the study it can be said that peroxidases confer antibiotic resistance to *C. chinensis* through prolonged insect development periods. Peroxidase activity may therefore be used as a biochemical marker for bruchid resistance in soybean. This finding has a practical application in that soybean varieties with higher peroxidase activity can be bred through genetic engineering (Dzhavakhiya and Shcherbakola, 2007).

The results indicate that several compounds in soybeans contribute to its anti-feedant and/or antibiotic effects against *C. chinensis.* However, it was clear from the study that nutritional factors were not responsible for variations in observed resistance. This is a significant finding since it implies that breeding for resistance to *C. chinensis* in soybean will not conflict with nutritional components for human consumption. However, there is further need to isolate these biochemicals and feed them to bruchids to determine if bruchids are directly affected by the biochemicals. In conclusion, secondary metabolites; peroxidase, tannin, and total antioxidants were the biochemicals associated with higher resistance to Callosobruchus chinensis in soybean while flavonoids were associated with higher susceptibility.

ACKNOWLEDGEMENTS

Authors are grateful to Intra ACP- Mobility Scheme (CSAA) for funding the first author's PhD study at Makerere University, Uganda. We thank the Carnegie Cooperation (New York), Next Generation Competitive Grant through RUFORUM for funding the research.

STATEMENT OF NO-CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

REFERENCES

Ahmed, D., Khan, M. and Saeed, R. 2015. Comparative Analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves. *Antioxidants* 4(2): 394–409. https://doi.org/10.3390/antiox4020394

Hodges, D. M., Delong, J. M., Forney, C. F., Prange, R. K., Delong, J. M., Hodges, D. M. and Prange, R. K. 1999. the Thiobarbituric anthocyanin for estimating lipid peroxidation in plant tissues containing and other interfering. *Planta* 207 (4): 604–611. https://doi.org/10.1007/s00425005052

Barbehenn, R. V. and Constabel, C. P. 2011. Tannins in plant-herbivore interactions. *Phytochemistry* 72 (13): 1551–1565. https://doi.org/10.1016/j.phytochem.2011.01.040

Dhole, V. J. and Reddy, K. S. 2016. Association of phytic acid content with biotic stress tolerance in mungbean (*Vigna radiata* L. Wilczek). *Phytoparasitica* 44 (2): 261–270.

Dzhavakhiya, V.G. and Shcherbakova L.A. 2007. Creation of disease-resistant plants by gene engineering. Studies in plant sciences https://doi.org/10.1016/B978-044452132-3/50021-3

Khan, K.M., Khan, A., Ishimoto, M., Kitamura, K. and Komatsu, S. 2003. Proteome analysis of the relationship between bruchid resistant and susceptible mungbean genotypes. *Plant Genetic Resources* 1 (2-3): 115-123. doi:10.1079/PGR200318

Kolawole, A. and Kolawole, A. 2014. Insecticides and bio-insecticides modulate the glutathione-related antioxidant defense system of cowpea storage bruchid (*Callosobruchus maculatus*). *International Journal of Insect Science* 6: 79–88 https://doi.org/10.4137/IJIS.S18029

Lattanzio, V., Lattanzio, V.M. and Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry*: *Advances in*

Research 661 (2): 23-67.

Lattanzio, V., Kroon, P.A., Quideau, S. and Treutter, D. 2008. Plant phenolics-secondary metabolites with diverse functions. pp. 1-24. In: Recent Advances in Polyphenol Research. Wiley-Blackwell Publishers.

Mahatma, M. K., Bhatnagar, R., Mittal, G. K. and Mahatma, L. 2011. Phenol metabolism in downy mildew resistant and susceptible genotypes of pearl millet. *Archives of Phytopathology and Plant Protection* 44 (7): 623–636. https://doi.org/10.1080/03235400903266404

Manisha, M. 2017. Secondary Metabolites : Meaning, role and types. *Australian Journal of Entomology* 2 (3): 111–122.

Minney, B. H. 1990. Breeding P*haseolus vulgaris* (common bean) for Resistance to the major pest Bruchids: Biochemical bases for seed resistance in wild lines. Durham University.

Mrudula, C. M. and Prabhu, A. A. 2014. Phytochemical quantification and antioxidant capabilities of Moringaoleifera, Basellaalba and Centellaasiatica leaf sources. *International Journal of Innovative Research in Science*, *Engineering and Technology* 3 (2): 9243–9251.

Msiska, U.M., Odong, T.L., Hailay, M., Miesho, B., Kyamanywa, S., Rubaihayo, P.R. and Tukamuhabwa, P. 2018. Resistance of Uganda soybean germplasm to Adzuki bean bruchid. *African Crop Science Journal* 26 (3): 399-415.

Nuwamanya, E., Patrick, R. R., Ssetumba, M., Samuel, K., Joseph, F. H. and Yona, B. 2014. Biochemical and secondary metabolites changes under moisture and temperature stress in cassava (*Manihot esculenta* Crantz). *African Journal of Biotechnology* 13 (31): 3173–3186. https://doi.org/10.5897/AJB2014.13663

Parthiban, V. K., V., P., and Prabakar, K. 2012. Changes in the biochemical constituents of carrot roots due to bacterial soft rot. *Pharmaceutical Technology* 1 : 231–238.

Shannon, L., Kay, E. and Lew, J. 1966. Horseradish Roots. *Journal of Biological Chemistry* 241 (9): 2166–2172.

Sharma, S. and Thakur, D. R. 2014a. Biochemical basis for bruchid resistance in cowpea, chickpea and soybean genotypes. *American Journal of Food Technology* 9 (6): 318–324. https://doi.org/10.3923/ajft.2014.318.324

Sharma, S. and Thakur, D. R. 2014b. Comparative developmental compatibility of *Callosobruchus maculatus* on cowpea, chickpea and soybean genotypes. *Asian Journal of Biological Sciences* 7(6): 270–276. https://doi.org/10.3923/ajbs.2014.270.276

Sharma, S. and Thakur, D. R. 2014c. Studies on the varietal preference of *Callosobruchus maculatus* on soybean genotypes. *Asian Journal of Biological Sciences* 2014: 1–5. https://doi.org/10.3923/ajbs.2014

Swain, T. 1977. Secondary compounds as protective agents. *Annual Reviews of Plant Physiology* 28: 479-501

Vijay, D. T. and Rajendra, S. 2014. Estimation of Total Phenol, Tannin, Alkaloid and Flavonoid. *Journal of Pharmacognosy* 2 (4): 41–47.

War, A. R., Murugesan, S., Boddepalli, V. N., Srinivasan, R. and Nair, R. M. 2017. Mechanism of resistance in mungbean [*Vigna radiata* (L.) R. Wilczek var. radiata] to bruchids, *Callosobruchus* spp. (Coleoptera: Bruchidae). *Frontiers in Plant Science* 8. https://doi.org/10.3389/fpls.2017.01031

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., and Sharma, H. C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior* 7(10): 1306–1320. https://doi.org/10.4161/psb.21663

Wong, S. K., Lim, Y. Y., and Chan, E. W. C. 2009. Antioxidant properties of Hibiscus: Species variation, altitudinal change, coastal influence and floral colour change. *Journal* *of Tropical Forest Science* 21 (4): 307–315.

Wu, K., Zhang, J., Zhang, Q., Zhu, S., Shao, Q., Clark, K. D. and Ling, E. 2015. Plant phenolics are detoxified by prophenoloxidase in the insect gut. *Scientific Reports* 5: 1–15. https://doi.org/10.1038/srep16823

Yahaya, I. A., Nok, A. J. and Bonire, J. J. 2013. Chemical studies of the Peel of Xanthosoma sagittifolium (Tannia Cocoyam). *Pakistan Journal of Nutrition* 12 (1): 40–44.

Zhu-Salzman, K., Luthe, D. S. and Felton, G. W. 2008. Arthropod-Inducible Proteins: Broad Spectrum Defenses against Multiple Herbivores. *Plant Physiology* 146 (3): 852–858. https://doi.org/10.1104/pp.107.112177