Journal of Basics and Applied Sciences Research (JOBASR)
ISSN print: 3026-9091
Volume 1(1) December 2023
DOI: https://doi.org/10.33003/jobasr-2023-v1i1-27

Antibiosis of Salicylic acid Pre-Hardening Treatments of Cowpea on Development of Pod Sucking Bug, *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae)

Audi, A. H.[1]* and Mukhtar, F. B.[2]
[1]Department of Biological Sciences, Faculty of Life Sciences, Bayero University, PMB. 3011, Kano
[2]Department of Plant Biology, Faculty of Life Sciences, Bayero University, PMB. 3011, Kano
*Corresponding Authors Email: audigenesis@yahoo.com, ahaudi.bio@buk.edu.ng

ABSTRACT
Induced plant response using Exogenous Salicylic acid has a significant potential to control physiological interactions of Phytophagous insects The experiment was conducted at the University Research farm, Faculty of Agriculture Bayero University, Kano (11°58 'N, 8° 25' E and 457m above sea level) to evaluate the effect of SA-treatment on Development of Pod sucking bug of cowpea. Four different cowpea varieties (IT97K-1069-6, IT98K-205-8, IT89KD-288 and Dan'ila) pre-hardened with Salicylic acid were established in various replicated field cages in completely randomized design. Five-pairs each of fresh bugs were introduced into the various cages. These were allowed for 2-weeks to mate and oviposit after which all adult insects are removed. Introduced insects showed variable developmental response to the different Pre-hardened cowpea varieties in SA hormones ($P<0.001$). Interactions of treatments and varieties was also found significant ($P<0.001$).Cowpea seeds treated with lower (5ppm) concentrations of SA show greater deleterious effects on the development of the pod bugs than the higher (10 & 20ppm) concentrations and the controls (Distilled water & Untreated seeds). Of the four Cowpea varieties screened, IT97K-1069-6 and IT89KD-288 of 5ppm SA-treatments show higher antibiosis on development of *C. tommentosicollis*, resulting to low oviposition (41.33 and 45.67), higher percentage nymphal mortalities (71.59% and 77.24%) and mean unhatched eggs of 27.33 and 32.00 respectively, with smaller adults bugs (3.55mg and 3.27mg) at emergence. Phytochemical screening of the treatments using GC-MS analysis showed high concentrations of cumene, eugenol and sesquiterpenes in the tolerant varieties. These relations should be explored extensively toward sustainable plant protection.

Keywords:
Cowpea varieties,
Salicylic acid,
C. tomentosicollis,
Control,
Phytochemicals.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp), (Leguminosae: Papilionidae) has been described as the most important legume crop and a major source of dietary protein in the tropical and sub-tropical regions of the world especially where availability of protein is low (Voster *et al.*, 2007; Singh and Singh, 2015; Devi *et al.*, 2015). However, a variety of insect pests frequently impede farmers' attempts to enhance the production of this valuable crop. *Clavigralla tomentosicollis*, a pod-sucking bug, is one such pest that causes significant economic loss. Both nymphs and adults suck the sap from the pods causing premature pod drying and shriveling (Jackai *et al.*, 2001). Because insect resistance made employing pesticides to control insects in pests ineffectual, the chemicals were applied at increasing doses to make up for their inefficiency. This is now a constant hazard to human health, the environment, and aquatic wildlife. It is highly desired to look for alternative approaches to productive and affordable cowpea production. Plants defend themselves both directly through physical and chemical defenses against herbivores and pathogens, and indirectly by recruiting natural enemies of herbivores (Aljbory and Chen, 2018). These direct and indirect defenses are regulated through biochemical pathways that rely on plant hormones to mediate physiological changes that aid in plant defense (Berens *et al.*, 2017). It is possible for both defense mechanisms to exist constitutively or to be activated upon injury by insects. One of the key elements of agricultural pest management is induced plant response, which has been used to control physiological interactions of Phytophagous insects.

Numerous studies reported that salicylic acid is a vital component of the plant signal transduction pathways causing disease and pathogen resistance (Maleck and Dietrich, 1999). Few studies have attempted to employ plant growth hormones exogenously as a surface spray to manage insect pests to cause a multitude of effects on the morphology and physiology of plants (Pancheva *et al.,* 1996). The SA plant defense responses are however characterized as species specific. This implied that, response of insect to plant Salicylates cannot be generalized but has to be tested for each plant-Insect combination (Sponsel and Hedden, 2004).

There have been prior reports of attempts to enhance plant resistance to insect attack in some plants by using plant growth hormones, such as salicylic acid (Aviv *et al.,* 2002; Brodersen *et al.,* 2002; Audi and Mukhtar, 2019; Lortzing *et al.,* 2019). Salicylic acids' (SA) impact on cowpeas' ability to withstand pod-sucking pests like *Clavigralla tomentosicollis*, however, has not received much attention. This research offers a unique approach to investigate the possibility of salicylic acid pre-sowing hardening treatments to induce resistance against pod bugs. The process of repeatedly soaking and hydrating seeds in a solution containing both organic and inorganic solutes is known as pre-hardening treatment. This method permits pre-germinative physiological processes while preventing radicle appearance. The aim of the research is to assess the deleterious effects of Salicylic acids (SA) treatment of cowpea seeds on the developmental stage of one of its notorious pod sucking insect, *Clavigralla tomentosicollis* ,

MATERIALS AND METHODS

The experimental field trials were conducted at the University Research farm, Faculty of Agriculture Bayero University Kano (11°58'N, 8°25' E and 457m above sea level. The mean annual rainfall was within the range of 865-1250mm with mean annual temperature of about 22-38°C and relative humidity of 65-90mmHg (Remote sensing unit Geography Dept, BUK).

Pre-sowing Hardening Treatments

Salicylic acids (SA) used were purchased from (Sigma Aldetch). Concentrations of the growth substances of salicylic acid were prepared in the laboratory by dissolving 1gram of each of Salicylic acid granules in 1ml of 75% ethanol for dilution in distilled water to make the stock solution (1000 ppm). These were then serially diluted to create salicylic acid concentrations of 5 ppm and 10 ppm. The seeds of the cowpea varieties (IT97K-1069-6, IT98K-205-8, IT89KD-288 and Dan'ila) were soaked into the 5ppm and 10ppm of Salicylic acids into 250mls labeled conical flasks for a period of 6 hours. These were shed dried in the laboratory before sowing (Darra *et al*., 1973; Audi and Mukhtar, 2019). In order to ensure that variations in seed development along with untreated seeds would not influence the impact of seed pretreatment on plant growth, distilled water was also utilized for both soaking and serving as a control (Darra *et al.,* 1973). The different Pre-hardened cowpea varieties were grown both in exposed field (normal unblocked/caged) to serve as a source of the insects and in in replicated field cages for the various experimental trials respectively (Tanzubil, 2000).

Insects Collection and Method of Infestation

Five pairs of fresh adult males and females pod sucking bugs *Clavigralla tomentosicollis* were collected from the exposed field using glass jars. This was carried out between 7:30 am and 9:00 am, when the insects were not as active for flying. Infestation was made during the late September (65-80days) after planting) when pod formation was in progress. Each set up was established in a 2-m by 4-m screen cage containing 6-stand of cowpeas planted in rows prevented from entrance by other insects from the main exposed planted cowpea crops. This ensures accurate assessment of infestation due to the test insects under study but prevent multiple infestations from other pests (Underwood *et al.,* 2002; Audi and Mukhtar, 2019).

Effect of pre-hardening treatments on oviposition of Pod sucking bug, *C. tommensicollis*

Plant resistance to insect pests was evaluated in terms of effects on the survival of the insects and of the plants reactions (Jackai *et al.,* 2001). Cowpea seedlings were infested with 5 pairs of adult males and females *C. tommensicollis* when pod formation was in progress. The insects were taken out of each plant after a week of being allowed to mate and lay eggs. On the underside of cowpea leaves, egg masses and clusters of reddish brown eggs were visible. From each randomly selected cowpea variety, these were tallied and the number of eggs laid per treatment was used to evaluate the insects' preference for oviposition (Underwood *et al.,* 2002).

Determination of Egg Hatchability and Nymphal Mortality of *C. tommensicollis*

The experimental setup for oviposition was kept up and observed in the several field cages until newly emerging nymphs were noticed. Eggs were assumed unhatched when they stayed firm when punctured with a pin or other sharp instruments, but the hatched eggs were observed to rupture. According to Ofuya and Osadahun (2005), nymphs were deemed dead if they remained still and unfazed by pinpricking. The percentage mortality of the nymphs was determined as expression of proportion of the cumulative eggs hatched multiplied by 100. The relative mortality of the nymphal bug was calculated as the difference between the total number of hatched eggs and the total number of adult bugs that survived the treatments

Determination of body weight of *C. tommensicollis*

Body weight at emergence is an indicator of resource use and nutrient availability of the host plant. Cultures were observed four weeks after infestation. Emerging *C. tommensicollis* adults were randomly collected into glass vials containing formalin-soaked cotton. A portion of the collected strangulated insects was counted and the average weight of 25 adult insects were determined in the field using a digital scale (Underwood *et al.*, 2002).This operation was repeated three times to obtain the average values and then the weighed insects were discarded**.**

Phytochemical Analyses

Fresh leaves and pods of different cowpea varieties were washed and dried in the shade at room temperature. The dried and ground plant fractions were weighed and extracted using 80% methanol in cold water (MeOH) supplemented with butylated hydroxy-toluene (BHT) as extraction solvent. Then, the extracts were quantitatively analyzed by gas chromatography-mass spectrometry GC-MS (QP 2010 Plus Shimadzu, Japan) at the National Research Institute of Chemical Technology (NARICT) Zaria. The relative quantity (%) of each component was calculated by comparing its average value peak area over the total area. The interpretation of the name, molecular weight, and compositional structure of the test materials were also determined by comparing the spectra of known constituents stored in the NIST library with the spectra of constituents under investigation (Valls *et al.*, 2009).

Statistical Analysis

All data collected by counting were subjected to square root transformation while percentages were arcsine transformed prior to analysis. Transformed data were subjected to Analysis of Variance ANOVA using Statistical Analysis and Experimental designusing SPSS software 20 (Version 08.12.14). Post hoc test was carried out using the Tukey test at 0.05 level of significance.

RESULTS AND DISCUSSION

The effect of pre-hardening treatments of cowpea on the oviposition of Pod sucking bug, *C. tommentosicollis* were presented in Table 1. The number of eggs laid by female insect differed significantly with variety type and hormone concentrations (P<0.001). The interaction of treatments and varieties was also found significant (P<0.001). Oviposition was considerably less in all hormone treated plants compared with distilled water treatments and the control (Table 1). The mean number of unhatched eggs was significantly higher at low concentrations (5 ppm), than those in the higher (10 & 20ppm) concentrations of SA treated cowpea and the controls with 3.75 and 2.67 for distilled water and untreated plants respectively. In response to treatment, cultivars IT97K-1069-6 and IT89KD-288-8 inhibited egg hatching to a greater extent than other cultivars with 27.12 and 32.00 unhatched eggs, respectively (Table 2).

The mean percentage nymphal mortality of *C. tommentosicollis* also differed significantly (P<0.001) between varieties and hormone concentrations. Similarly, higher mortality was observed in different hormone treatments. Regarding the effect between treatments, mortality of developing nymphs was significantly higher (69%) in the 5 ppm SA treatment than in the 10 and 20 ppm SA treatments with 54.95% and 42.16% and respectively The response of varieties to treatments showed significantly higher percent nymphal mortality in the 5 ppm SA treatment of IT97K-1069-6 and IT89KD-288 varieties, 71.59% and 77.24%, respectively. The average weight of adults bug at spikelet emergence also showed a similar trend (Table 4).

The relative proportion of secondary metabolites produced by different treatments of cowpea also varied significantly (P<0.001) with hormone concentration. Pretreatment of cowpea seeds with SA induced the synthesis and production of insecticidal compounds in IT97K-1069-6 and IT89KD-288 seeds, which increased their resistance (Table 5). The concentrations of terpenes, phenols and tannins were significantly higher (P<0.001) in SA hormone treatment at a concentration of 5 ppm compared to other treatments and control.

Table 1: Effect of Salicylic acid (SA) Pre-hardening Treatments on Oviposition of *C. tommentosicollis*

Hormone treatments	No. of Cowpea stands	Cowpea Varieties				Treatment Effects
		IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	6	41.33	45.67	75.00	76.33	59.58
10ppmSA	6	50.67	82.00	71.67	89.33	73.42
20ppmSA	6	70.35	99.02	71.44	91.18	83.00
DIST. H_2O	6	95.33	91.00	93.33	98.67	94.58
Untreated	6	88.00	100.00	101.33	107.00	99.08
MEAN	6	69.14	83.54	82.55	92.50	81.93
$LSD_{5\%}$	6	2.45	2.87	3.62	4.07	

Mean values with differences less than LSD values at 5% are not significantly different, P<0.001

Table 2: Mean Number of Unhatched Eggs of *C. tommentosicollis on different* Salicylic acid (SA) Treated *Cowpea*s

Hormone treatments	No. of Cowpea stands	Cowpea Varieties				Treatment Effects
		IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	6	27.12	32.00	26.00	22.00	26.78
10ppmSA	6	25.67	23.33	25.67	27.67	25.59
20ppmSA	6	13.27	15.33	11.00	9.00	12.15
DIST. H_2O	6	6.33	1.67	5.67	1.33	3.75
Untreated	6	3.00	1.67	4.00	2.00	2.67
MEAN	6	15.08	14.80	14.47	12.40	14.19
$LSD_{5\%}$	6	1.57	1.42	1.56	1.35	

Mean values with differences less than LSD values at 5% are not significantly different, $P<0.001$

Table 3: Effect of Salicylic acid (SA) Pre-hardening Treatments on Nymphal Mortality (%) of *C. tommentosicollis*

Hormone treatments	No. of Cowpea stands	Cowpea Varieties				Treatment Effects
		IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	6	71.59	77.24	64.60	64.98	69.60
10ppmSA	6	50.04	59.92	57.13	52.72	54.95
20ppmSA	6	40.12	39.05	43.14	46.32	42.16
DIST. H_2O	6	5.01	2.37	6.22	2.95	4.14
Untreated	6	2.59	1.27	2.83	2.40	2.27
MEAN	6	33.87	35.97	34.78	33.87	34.62
$LSD_{5\%}$	6	1.49	1.45	1.55	1.89	

Mean values with differences less than LSD values at 5% are not significantly different, $P<0.001$

Table 4: Effect of Salicylic acid (SA) Pre-hardening Treatments on Mean Weight of Adult Bugs (mg) of *C. tommentosicollis*

Hormone treatments	No. of Cowpea stands	Cowpea Varieties				Treatment Effects
		IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	6	3.55	3.27	4.65	4.13	3.90
10ppmSA	6	4.13	3.96	4.21	4.65	4.24
20ppmSA	6	4.37	4.53	4.83	4.97	4.68
DIST. H_2O	6	5.59	5.73	5.89	5.47	5.67
Untreated	6	5.97	5.56	5.87	6.05	5.86
MEAN	6	4.72	4.61	5.09	5.05	4.87
$LSD_{5\%}$	6	3.44	3.39	3.50	3.46	

Mean values with differences less than LSD values at 5% are not significantly different, $P<0.001$

Table 5: Relative Concentration of Phytochemicals Detected from different Pre-hardened Cowpea Varieties using Gas Chromatography Mass Spectroscopy (GC-MS) Spectrum Comparison

Hormone Treatments	Cowpea Varieties											
	IT97K-1069-6			IT89KD-288			IT98K-250-8			DAN' ILA		
	RT	**MC**	**Phytochemicals**	**RT**	**MC**	**Phytochemicals**	**RT**	**MC**	**Phytochemicals**	**RT**	**MC**	**Phytochemicals**
5ppm SA	19.64	15.49	Dimethylbenze (monoterpenes)	3.51	17.83	Quinolines (Tannin) , Methyl hexane	3.54	7.12	1, 2-Dimethylbenzene, o-Methyltoluene	4.95	6.64	Ethylcyclo haxane, Isobutyl cyclohexane
	19.64	9.17	Dihydrogeraniol	3.51	9.83	Phenylethane	3.51	8.03	Cumol, Pseudocumol	4.95	6.67	Octadecadienol, Phenylethane
	19.64	7.49	2-hydroxyethoxyethyl (Phenolic)	3.51	6.83	Ethylbenzol	3.51	9.03	Pentadecanecarboxyli c acid, ethylbenzol	4.95	6.15	Ethylbenzol
10ppm SA	22.36	24.71	Phytol (Diterpene, Cyclohexane.	23.78	28.28	n-Hexadecanoic acid, Isobutylcyclohe xane	22.16	7.23	1, 2-Dimethylbenzene, o-Methyltoluene	3.58	4.09	Dimethylbenzene , cyclohexane
	22.36	13.68	2-hydroxyethoxyethyl (Eugenol)	23.78	13.74	Pentadecanecar boxylic acid, ethylbenzol	22.16	8.09	Pentadecanecarboxyli c acid	3.51	6.50	Ethylbenzol and Pentadecanecarb oxylic
	22.36	15.55	Quinolines (Tannin) Iodomethylbenzoic.	23.78	14.88	Phenylethane, Octadecadienol, quinoxalin (Phenolic)	22.16	8.09	Phenylethane	3.58	7.19	Octadecadienol
D.H_2O	22.30	3.57	Trimethyl benzene	3.82	3.49	Methyltoluene,	22.38	5.96	o-Methyltoluene	3.66	2.82	Dimethylbenzene
Untreated	22.33	4.69	Methyltoluence	26.55	2.11	Methyleicosane	22.36	4.54	o-Methyltoluene	21.23	2.91	1, 2-Xylene

Discussions

Cowpeas pre-hardened with varying salicylic acid concentrations responded differently to attacks by pod-sucking bugs. Antibiosis, or the harmful effects of treatments on insect development in terms of decreased oviposition, larval mortality, failure of egg to hatch, and smaller insects at emergence, is primarily responsible for cowpea resistance to the pod sucking bug infestation, *C. tommentosicollis* (Jackai, *et al.*, 2001). It was discovered that there were notable differences in the oviposition of *C. tommentosicollis* between the various treated cowpea varieties and the controls.

The results of the various treatments indicated that, 5ppm SA was superior to the other treatments in terms of lowering bug oviposition. This is explained by the treated cowpeas' increased activation of the plant defense response, which may have set off reaction pathways that produced a variety of essential metabolite combinations (n-Hexadecanoic acid and Cyclohexane) that prevented the bugs from laying a significant number of eggs in the less infested varieties (Table1). This is consistent with research by De Boer *et al.* (2004), which showed that MeSA released during infestation inhibited the oviposition of cabbage moths, *Mamestra brassicae* L., indicating that MeSA may also be detected by the herbivores that are attacking the plants. Pre-hardening treatments of IT97K-1069-6 and IT89KD-288 in the lower (5ppm SA) concentration reduced the number of eggs laid dramatically, but they also made the eggs less viable for *C. tommentosicollis* to hatch.

The number of eggs laid was drastically reduced by pre-hardening treatments of IT97K-1069-6 and IT89KD-288 in the lower (5ppm SA). However, similar treatments also affected the hatchability of eggs from *C. tommentosicollis*, resulting in a higher number of unhatched eggs than in higher (10&20ppm SA) concentrations (Table 2). This could be related to the metabolic production of high levels of phenolic compounds found in their leaves (Table 5), which are likely caused by enzyme systems like aldehyde oxidases and salicylic acid signals that reduce hatchability (Divol *et al.*, 2005).

Additionally, pre-hardening treatments of IT97K-1069-6 in 5 ppm SA led to a high mortality rate of *C. tommentosicollis* nymphs. When the developing nymphs on IT97K-1069-6 were examined closely, they revealed increased retention in the dark/black colored surface cuticle and reduced activity. The developing nymphs died as a result of the body shrinking and appearing to be somewhat dehydrated, with the exception of a small number of nymphs that moulted and turned yellowish brown. Quinolé production and phytols found in the leaves and pods of the cowpea varieties IT97K-1069-6 and IT89KD-288 may be related to this. Similar research by Maffei *et al.* (2007) revealed that salicylates, a type of simple phenolic, function as an antifeedant to insect herbivores and that there is a negative correlation between salicylate levels and *Operophtera brumata* (L.) nymphal growth in Salix leaves. Pieterse and van Loon (1999) reported that salicylic acid (SA) may trigger the synthesis of a regulatory protein called Non-Expressor of Pathogenesis-Related genes (NPR1), which can be translocated through the leaves vascular system and act as an antifeedant to insect herbivores. This could explain the similar effect of the cowpea varieties IT97K-1069-6 and IT89KD-288 pre-hardened in SA to the developing nymphs of *C. tommentosicollis*, leading to higher insect mortality.

CONCLUSION

Salicylic acid pre-hardening treatments for cowpeas had a significant impact on a number of regulatory responses to the pod-sucking bug, *C. tommentosicollis*. Low (5 ppm) concentrations of SA on pre-hardened cowpea exhibit more detrimental effects on the insects than the higher (10 ppm) concentrations and controls. Pre-hardening treatments of IT97K-1069-6 and IT89KD-288 demonstrated increased antibiosis to the bug's development, leading to a higher percentage of nymphal mortalities and unhatched eggs as well as a notable decrease in adult *C. tommentosicollis* weight. However, in the IT98K-205-8 and Dan'ila varieties of the same treatments and controls, the percentage of nymphal mortality and unhatched eggs was significantly lower. The tolerant varieties had high concentrations of cumene, eugenol, and sesquiterpenes, according to phytochemical screening of the treatments conducted using GC-MS analysis. Further research on these relationships is necessary to protect plants in a sustainable manner.

ACKNOWLEDGEMENT

The authors acknowledged Facility support from Department of Biological Sciences, Pure and Applied Chemistry, Bayero University and National Research Instititute for Chemical Technonlogy (NARICT), Zaria.

REFERENCES

Aljbory1, Z and Chen, M.(2018). Indirect plant defense against insect herbivores: a review. Insect Science, 25 (1):2-23. doi: 10.1111/1744-7917.12436

Audi, A. H. and Mukhtar, F. B. (2019). Evaluation of Salicylic acid Pre-Hardening Treatments of Cowpea for Resistance against the Flea Beetle, Podagrica fuscicornis Linn. (Coleoptera: Chrysomelidae) International Journal of Environment, Agriculture and Biotechnology, 4 (1):33-40.

Aviv, D. H., Rusterucci, C., Holt, B. F., Dietrich, R. A., Parker, J. E. and Dangl, J. L. (2002). Runaway cell death, but not basal disease resistance, in lsd1 is SA- and NIM1/NPR-dependent. *Plant Journal*, 29: 381–391.

Berens, Matthias L, Berry, H. M., Mine, A., Argueso, C. T., Tsuda, K. (2017). Evolution of Hormone Signaling Networks in Plant Defense. *Annual Review Phytopathology,* 4:55:401-425 doi: 10.1146/annurev-phyto-080516-035544

Brodersen, P., Petersen, M., Pike, H. M., and Olszak, B. (2002). Knockout of *Arabidopsis* accelerated-cell-death11 encoding as phingosine transfer protein causes activation of programmed cell death and defense. *Genes and Development*, 16: 490–502. doi:10.1101/gad.218202

Darra, B. L., Seith, S. P., Singh, H. and Mendiratta, R. S. (1973). Effects of hormone directed presoaking emergence and growth of osmotically stressed wheat (*Triticum aestivum* L) -seeds. *Agronomy Journal,* 65: 295-299. doi:10.5958/2319-1198.2018.00022.2

De Boer, J. G., Posthumus, M. A. and Dicke, M. (2004). Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. *Journal of Chemical Ecology,* 30:22-30.bv doi: 0098-0331/04/1100-2215/0

Devi, C.B., Kushwaha, A. and Kumar, A. (2015). Sprouting characteristics and associated changes in nutritional composition of cowpea (Vigna unguiculata). Journal of Food Science Technology, 52 (10), 6821–6827. doi:10.1007/s13197-015-1832-1

Divol, F., Vilaine, F., Thibivilliers, S., Amselem J, and Palauqui, J. C. (2005). Systemic response to aphid infestation by *Myzus persicae* in the phloem of *Apium graveolens*. *Plant Molecular Biology*, 57: 517–540. doi/10.1007/s11103-005-0338-z

Jackai, L.E.N., Nokoe, S., Tayo, B.O. and Koona, P. (2001). Interferences on pod wall and seed defenses against the brown cowpea coreid bug, *Clavigralla tomentosicollis* Stal. (Hem., Coreidae) in wild and cultivated *Vigna* species. *Journal of Applied Entomology*, 125, 277–286. doi: 10.1603/0022-0493-95.6.1281

Lortzing, V., Oberländer, J., Lortzing, T. Tohge, T., Steppuhn, A., Kunze, R., Hilker, M. (2019). Insect egg deposition renders plant defence against hatching larvae more effective in a salicylic acid-dependent manner *Plant Cell and Environment*, 42 (3): 1019-1032 doi: 10.1111/pce.13447

Maffei, M.E., Mithofer, A. and Boland, W. (2007). Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry,* 68:46-59. doi.org/10.1016/j.phytochem.2007.07.016

Maleck, K. and Dietrich, R. A. (1999). Defense on multiple fronts: how do plants cope with diverse enemies? *Trends Plant Science*, 4: 215–219. doi:https://doi.org/10.1016/S1360-1385 (99)01415-6

Ofuya, T. I. and Osadahun, J. M. (2005). Effect of three Different Powders on Behaviour, Mortality and Reproductive Fitness of *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Zoological Research*, 26(6):603-608.

Pancheva, T.V. and Popova, L. P., Uzunova, A. N. (1996). Effects of salicylic acid on growth and photosynthesis in barley plants. *Journal of Plant Physiology*, 149, 57–63. doi.org/10.1016/S0176-1617 (96) 80173-8

Pieterse, C.M.J. and van Loon, L.C. (1999). Salicylic acid-independent plant defence pathways. *Trends in Plant Science,* 4: 52–58. doi.org/PII: S1360-1385(98)01364-8

Singh, C. and Singh, N.N. (2015). Bioefficacy and economics of different insecticides again legume pod borer (Maruca vitrata Fabricius) infesting cowpea (Vigna unguiculata Walpers). *Indian Journal of Agricultural Resources*, 49 (4), 358–362. doi : 10.5958/0976-058X.2015.00065.7

Sponsel, V. M. and Hedden, P. (2004). Gibberellin biosynthesis and inactivation of Plant hormone biosynthesis, signal transduction. Action. Davies P. J. Ed: Part B, Kluwer Academic. Publ. Dordrecht, 63-94pp. doi: 10.1104/pp.105.064162

Tanzubil, P. B. (2000). Field evaluation of Neem extracts for control of insect pests of cowpea in Northern Ghana. *Journal of Tropical Forest Products* (Malaysia) 6: 165 – 172. doi:10.5555/20013089506

Underwood, N., Rausher, M. D. and Cook, W. (2002). Bio-assay versus chemical assay: measuring the impact of induced and constitutive resistance on herbivores in the field. *Oecologia*, (Berlin) 131:211–219. doi 10.1007/s00442-002-0867-y

Valls, J., Millán, S., Martí, M.P., Borràs, E. and Arola, L. (2009). Advanced Separation Methods of Food Anthocyanins, Isoflavones and Flavanols. *Journal of Chromatography A*, 1216, 43: 7143-7172. doi.org/10.1016/j.chroma.2009.07.030

Voster, I. H. J., Jansen, V. R. W., VanZijl, J. J. B. and Venter, S. L. (2007). The importance of traditional leafy vegetables in South Africa. *African Journal of Food, Agric. Nutritional Development,* 7(4): 1-12. doi: 10.5897/AJAR09.724