

THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Utilization of Salicylic acid Pre-Hardening Treatments for the Control of Pod Sucking Bug, *Clavigrallatomentosicollis* Stal (Hemiptera: Coreidae)

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Abstract:

The experiment was conducted at the Agric. Research farm Bayero University Kano (11°58' N, 8° 25' E and 457m above sea level) to evaluate the potential of SA-treatment in the control of Pod sucking bug of cowpea. 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. 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 pre-mated *Clavigrallatomentosicollis* were introduced into the various cages, allowed for 2-weeks to mate and oviposit after which all adult insects are removed. The different cowpea varieties screened showed variable response to the bugs attack ($P < 0.001$). Cowpea varieties IT97K-1069-6 and IT89KD-288 recorded low indices of susceptibility and damage to pod bug attack, delayed development time of nymphal bugs with few progeny emergence. Pre-treatment effects also varied significantly with hormone concentrations ($P < 0.001$) with 10ppm Salicylic acid (SA) being more effective than the lower (5ppm) concentrations and the controls. The interaction of treatments and varieties was also found significant ($P < 0.002$). Of the four Cowpea varieties screened, IT97K-1069-6 and IT89KD-288 pre-hardened with high (10ppm SA) concentration of SA were found tolerant owing to their lower susceptibility indices (9.58 and 9.26) and mean percentage pod damage levels of 19.97% and 24.51% respectively, while Dan'ila and IT98K-205-8 of the same treatments were however susceptible. Phytochemical analysis of the treatments showed appreciable 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, control, photochemical, *clavigrallatomentosicollis*

1. 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 (Johnson et al., 1983; Anderson, 1985; Ofuya, 1986; Opara et al., 1998; Voster et al., 2007). However efforts to maximize production of this valuable crop by farmers are often hampered by myriads of insect pests of which damage due to pod sucking bug, *Clavigrallatomentosicollis* causes huge economic loss. Control of insect pests using pesticides was ineffective as a result, the chemicals are used at increasing dose in order to compensate for its ineffectiveness due to insect resistance. This has become a continuous threat to aquatic fauna, environmental health and human health. The search for alternative approach towards efficient and cost-effective means of production of cowpea is very desirable. Plants respond to herbivore attack through an intricate and dynamic defense system that includes structural barriers, toxic chemicals, and attraction of natural enemies of the target pests (Hanley et al., 2007; Howe and Jander, 2008; Karban, 2011). Both defense mechanisms (direct and indirect) may be present constitutively or induced after damage by the herbivores. Induced response in plants is one of the important components of pest control in agriculture, and has been exploited for regulation of insect's herbivore population (Howe and Jander, 2008; Sharma, 2009; Agrawal, 2011). This physiological interaction of plants and insects has resulted in the development of an elegant defense system in plants that has the ability to recognize the non-self-molecules or signals from damaged cells, much like the animals, and activates the plant immune response against insects' herbivores (Howe and Jander, 2008; Hare, 2011).

Numerous studies argue that salicylic acid is a vital component of the plant signal transduction pathways causing disease and pathogen resistance (Maleck and Dietrich, 1999). Exogenous applications of SA, either by direct injection or by spraying, have been reported to cause a multitude of effects on the morphology and physiology of plants (Pancheva et al., 1996). The SA involved plant defense responses are characterized as species specific. Even in two phylogenetic closely related plant species such as tomato and tobacco, the SA-dependent defense pathway does not trigger the same defense responses. It also means that the outcome of a Salicylic acid treatment cannot be predicted and has to be tested for each

plant-Insect combination (Sponsel and Hedden, 2004). Previous attempt to use plant growth hormones such as salicylic acid (Alvarez, 2000; Aviv et al., 2002; Brodersen et al., 2002; Walling, 2000) to modify plant tolerance to insect attack have been reported in some plants. However, little was found on how Salicylic acids (SA) affect resistance of cowpea to pod sucking bugs *Clavigrallatomentosicollis* while most research uses plant growth hormones exogenously as surface spray to control insect pests. This research takes a new dimension to test the potential of Salicylic acid to induce resistance against pod bugs using pre-sowing hardening treatments. It is the repeated soaking and seed hydration in a solution of organic and inorganic solutes which allow pre-germinative physiological activities but prevent radicle emergence.

2. 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), From June-November, 2014. 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).

2.1. Pre-sowing Hardening Treatments

Different concentrations (5ppm and 10ppm) each of the growth substances of salicylic acid was 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 (1000ppm). These were subsequently diluted to various concentrations 5ppm and 10ppm of salicylic acids which were transferred from the reagent bottles into clearly labelled 250mls conical flasks according to the concentration of the growth substances to be used in the pre-hardening treatments. The seeds of the cowpea varieties (IT97K-1069-6, IT98K-205-8, IT89KD-288 and Dan'ila) were soaked in the various concentrations (5ppm and 10ppm) of Salicylic acids for a period of 6 hours. These were air dried in the laboratory before sowing (Darra et al., 1973; Audi and Mukhtar, 2009). Distilled water was also used for soaking and to also serve as control (Darra et al., 1973) so that the effect of seed pretreatment on plant growth should not be affected by the differences in seed development along with untreated seeds for comparing the effect of various pre-treatments.

2.2. Sowing of Cowpea Seeds

Four different cowpea varieties (IT97K-1069-6, IT89KD-288, IT98K-205-8, and Dan'ila) Pre-hardened with salicylic acid were grown both in exposed field (normal unblocked/caged) and various replicated field cages.

The field cages were for the screening against pod sucking bugs while the exposed field grown cowpea were used to monitor infestation, yield determination, Insect/predators relationship and also serve as a source of the insects used for the various field cage experiments. The seeds were sown into four replicates on a split plot designed in a completely randomized block design, with level of hormone treatments representing the main plots as well as the controls while the four varieties stand as sub plots (field cages) in each main plot. A spacing of 75x20cm inter and intra row were used respectively and 4-seeds were placed per hole and thinned to 2 seedlings per stand at 2-weeks after sowing (Tanzubil, 2000).

2.3. Insects Collection and Method of Infestation

Five pairs of adult males and females of freshly pre-mated pod sucking bugs *Clavigrallatomentosicollis* 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 relatively less active and would not readily take to flight when disturbed. Infestation was made during the late September (65-80days after planting) when pod formation was in progress. Each set up was established in 2-m by 4m screen cage with a door opening to the outside above a 0.5-m sill (which helped to contain walking nymphal bugs and the adults). In each cage, 6-stand of cowpea were planted in rows prevented from entrance by other insects from the main exposed planted cowpea crops this ensure accurate assessment of infestation due to the test insects under study but prevent multiple infestation from other pests. The insects were allowed to mate and lay eggs for a period of 2weeks after which they were removed from each plant (Underwood et al., 2002).

2.3.1. Effect of Pre-Hardening Treatments on Progeny Emergence and Developmental Time of *C. Tomentosicollis*

To determine progeny emergence of *Clavigrallatomentosicollis*, the above set up were maintained undisturbed but observed regularly until nymphs emerged. These were counted and discarded daily until no further progeny emergence observed. The total number of insects that emerged over a developmental period was determined by count for each treatment (Underwood et al., 2002). The mean developmental period of *C. tomentosicollis* was estimated as time from the middle of the oviposition period to the emergence of 50% of the F1 generation.

2.3.2. Effect of Pre-Hardening Treatments on Pod Damage By Pod Sucking Bug, *C. Tomentosicollis*

A similar set up was maintained unperturbed but allowed the nymphs to develop through five instars with morphological changes while feeding by sucking the sap of pods over 4 weeks periods until adult bugs emerged. Visual Pod damage caused by nymphal bugs during period of exposure was determined based on scale rating by (Heinrich et al., 1985; Jackai and Singh, 1988) using the following formula as:

$$\text{Percentage of Damaged Pods} = \frac{\text{Number of Damaged Pods Per Plant}}{\text{Total Number of Pods Per Plant}} \times 100$$

$$SI = \frac{\text{LogeY} \times 100}{D}$$

2.3.3. Determination of Susceptibility Indices

Susceptibility of the different cowpea cultivars to the bug attack was evaluated based on the cumulative nymphal emergence count of *F₁C. tomentosicollis* to their mean developmental time on various treatments, by Dobie (1974) as follows:

Where;

SI = Susceptibility index

Y = total number of emerged adults

D = Mean development period of the progeny

2.4. Phytochemical Analyses

Fresh Leaves and pods of the different cowpea varieties were washed and shade dried at room temperature. The dried and grounded plant part were weighed and extracted and using 80% cold aqueous methanol (MeOH) supplemented with butylated hydroxy-toluene (BHT) as an extracting solvent. Extracts were further subjected to Quantitative analysis at National Research Institute for Chemical Technology (NARICT) Zaria, using Gas Chromatography Mass Spectrometry GC-MS (QP 2010 Plus Shimadzu, Japan). The relative % amount of each component was calculated by comparing its average peak area to the total areas. Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were determined by comparing spectrum of the known component with that of the known components stored in the NIST library (Vallset al., 2009).

2.5. 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 the Genstat Statistical Software (2011), version 10.3DE, Rothamsted Experimental Station.

3. Results

Table 1 shows the effect pre-hardening treatments on development of pod sucking bug, *Clavigrallatommentosicollis*. The mean nymphal emergence varied significantly among varieties and hormone concentrations ($P < 0.001$). Interactions of treatments and varieties were also found significant ($P < 0.001$). Nymphal Progeny emergence was less in the 10ppmSA hormone treated cowpea seeds (40.75) than the 5ppmSA. But the emergence was considerably higher in both the distilled water treatment (83.66) and the control (89.17). Development time was observed to be significantly longer ($P < 0.001$) in all the hormone treatments when compared with the controls (Table 1). Nymphal development time was shorter (27.08 days) in the 5ppmSA treatments than the higher (10ppmSA) concentration (35.75 days). Varietal response to treatments shows that IT97K-1069-6 and IT89KD-288 of 10ppmSA pre-hardening treatments prolonged developmental time of *C. tomentosicollis* with lower numbers of nymphs (37.00 and 38.00) respectively compared with IT98K-205-8 and DAN'ILAVARIETIES from same treatments.

Hormone Treatments	Cowpea Varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
Mean Numbers of Nymphal Emergence					
5ppmSA	55.67	41.00	55.67	43.67	49.00
10ppmSA	37.00	38.00	37.00	51.00	40.75
DIST. H ₂ O	95.33	63.33	69.00	107.00	83.66
CONROL	91.00	70.00	80.67	115.00	89.17
MEAN	69.75	53.08	60.58	79.17	65.65
LSD _{5%}	15.610	8.854	10.398	20.533	13.489
Nymphal Developmental time (Days)					
5ppmSA	32.00	25.67	28.33	22.33	27.08
10ppmSA	39.33	30.00	37.67	36.00	35.75
DIST. H ₂ O	27.33	21.00	24.33	22.33	23.75
CONROL	17.67	24.33	20.67	21.67	21.08
MEAN	29.08	25.25	27.75	25.58	26.92
LSD _{5%}	5.031	2.066	4.057	3.855	3.538

Table 1: Effect of Salicylic Acid (SA) Pre-Hardening Treatments on Nymphal Development of *C. tomentosicollis* Mean Values with Differences Less than the Least Significant Differences (LSD) at 5% are Not Significantly Different, $P < 0.001$

Table 2 shows the response of the different pre-hardened cowpea varieties to attack by pod sucking bug, *Clavigrallatommentosicollis*. The tolerance capacity in terms of susceptibility index and pod damage levels also varied significantly ($P < 0.001$) with variety type and Salicylic acid hormone concentrations. The interaction of treatments and varieties was also found significant ($P < 0.002$). Susceptibility index and mean percentage pod damages were remarkably

lower in the entire hormone treated cowpea seeds compared with the distilled water treatments and the control. Treatment with 10ppmSA resulted in significantly ($P<0.001$) lower susceptibility indices and pod damages (10.30 and 33.86%) when compared with 5ppmSA (14.43 and 42.56%) and controls. Varietal response shows that IT97K-1069-6 and IT89KD-288 of 10ppmSA pre-hardening treatments were tolerant with 9.58 and 9.26 susceptibility indices and mean percentage pod damages of 19.97% and 24.51% respectively while IT98K-205-8 and Dan'ila varieties from same treatments were however susceptible (Table 2).

Hormone Treatments	Cowpea Varieties				Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
Susceptibility indices (SI)					
5ppmSA	14.19	11.64	17.16	14.73	14.43
10ppmSA	9.58	9.26	9.28	13.08	10.30
DIST. H ₂ O	18.87	15.19	18.97	22.28	18.83
CONROL	21.77	24.10	20.44	19.52	21.46
MEAN	16.10	15.05	16.46	17.40	16.25
LSD _{5%}	2.969	3.609	2.758	2.355	2.726
Mean Percentage Pod Damage (%)					
5ppmSA	28.26	33.13	53.49	55.37	42.56
10ppmSA	19.97	24.51	45.86	45.09	33.86
DIST. H ₂ O	84.02	76.55	75.92	89.74	81.56
CONROL	75.04	67.92	83.27	71.48	74.43
MEAN	51.82	50.53	64.63	65.42	58.10
LSD _{5%}	17.958	14.173	9.876	10.823	12.990

Table 2: Effect of Salicylic Acid (SA) Pre-Hardening Treatments on Susceptibility and Pods Damage of Cowpea Byc. *Tomentosicollis*

Mean values with differences less than the Least significant Differences (LSD) at 5% are not significantly different, $P<0.001$

The relative proportion of secondary metabolites produce from different treated cowpea plants varied significantly ($P<0.001$) with hormone concentrations. Pre-hardening treatment of cowpea with SA elicited synthesis and production of chemical compounds with insecticidal effect that enhance tolerance capacity in some of the treated seeds than the controls (Table 3).

Treatments	Hormone 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	Methyltoluene, Methyl hexane	3.54	7.12	o-Methyltoluene	4.95	6.64	Isobutyl cyclohexane
	19.64	9.17	Dihydrogeraniol	3.51	9.83	Phenylethane	3.51	8.03	Cumol, Pseudocumol	4.95	6.67	Octadecadienol, Phenylethane
10ppm SA	19.64	7.49	2-hydroxyethoxyethyl (Phenolic)	3.51	6.83	Ethylbenzol	3.51	9.03	Pentadecanecarboxylic acid.	4.95	6.15	Ethylbenzol
	22.36	24.71	Phytol (Diterpene, Cyclohexane.	23.78	28.28	n-Hexadecanoic acid, Isobutylcyclohexane	22.16	7.23	o-Methyltoluene	3.58	4.09	Dimethylbenzene, cyclohexane
	22.36	13.68	2-hydroxyethoxyethyl (Eugenol)	23.78	13.74	Pentadecanecarboxylic acid, ethylbenzol	22.16	8.09	Pentadecanecarboxylic acid	3.51	6.5	Pentadecanecarboxylic
D.H ₂ O	22.36	15.55	Quinolines (Tannin) Iodomethylbenzoic.	23.78	14.88	Octadecadienol, quinoxalin (Phenolic)	22.16	8.09	Phenylethane	3.58	7.19	Octadecadienol
	22.3	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	Methyltoluene	26.55	2.11	Methyleicosane	22.36	4.54	o-Methyltoluene	21.23	2.91	1, 2-Xylene

Table 3: Relative Proportion of Phytochemicals Detected from Different Pre-Hardened Cowpea Varieties Using Gas Chromatography Mass Spectroscopy (GC-MS)

4. Discussion

Pre-hardening treatments of cowpea in various concentrations of salicylic acids resulted in varied responses to pod bugs attack. Cowpeas treated with 10 ppm SA were more tolerant to *C. tomentosicollis* attack than at lower concentrations (5 ppm). The population of the bugs was considerably high in control of IT98K-205-8 and Dan'ila due to high nymphal emergence but was low in the less susceptible varieties (Table 1). The developmental time of the bugs was

longer in the 10ppmSA treatments of IT97K-1069-6 and IT89KD-288 than the other treatments and controls in which the shortest development time was observed in Dan'ila variety. This prolonged developmental period with fewer nymphal emergences are consequent of effects of antifeedants cell sap in the pods of the less damage varieties which are mainly tannins (2-hydroxyethoxyethyl) and phenolic derivatives (Octadecadienol). This can be related to the findings of Barakat et al. (2010) and War et al. (2011) who reported that, insect attack may cause variable alterations in plant phenols which may elevate the activities of oxidative enzyme in response thereby triggering synthesis of defensive compounds.

Varietal response to treatments revealed that IT97K-1069-6 and IT89KD-288 of 10ppmSA pre-hardening treatments were more tolerant to attack by pod sucking bugs than with other treatments in which Dan'ila variety was more prone to infestation and pod damage owing to their high index of susceptibility (Table 2). Susceptibility Index is a measure of crop resistance, higher index of susceptibility implied that more progeny developed from a variety over a shorter time. The low susceptibility indices of IT97K-1069-6 and IT89KD-288 treatments could be attributed to higher levels of glucosinolates (Butylhydrogenphthalate and Myricic acid) and phenolic derivatives (2-hydroxyethoxyethyl) in them which may have adversely affect utilization of nutrients. A similar finding was earlier reported by Barbehenn and Peter (2002) who revealed that, ingested tannins reduce the digestibility of the proteins which decreased nutritive value of plants and plant parts to herbivores.

The mean percent pod damage was considerably low in the 10ppmSA treatments of the different cowpea varieties than the lower concentrations. Pod damages were relatively high in the treatments of IT98K-205-8 and Dan'ila compared with other varieties. A characteristic constriction and shriveling of pods which resulted to premature drying was irregularly observed to varying degrees. Crops with such damage show reduce pod productions and thus sustained fewer pods per stand which resulted to low yield or total crop failure. The recovery potentials of 10ppmSA pre-hardened cowpea varieties from pod sucking bug attack was higher than the lower concentration which when damaged, doesn't show enhanced recovery capacity even in the tolerant varieties but the crops are mostly lost by drying prematurely. This could be attributed to high accumulation of oxilipins mostly linoleic acid detected (Table 3) which might have played a significant role in rapid growth response that replenished damage tissues of the 10ppmSA treated cowpeas which made the plant tolerant despite attack by insect pests (Price, 1991). This is also consistent to the report of Creelman and Mullet (1997) and Li et al. (2002) which shows that Linoleic acid usually released by damaged cell membrane lipids can be converted enzymatically to jasmonic acid which had been implicated with jasmonate-octadecanoid signaling pathway essential for plant induced defense against chewing cell content-feeding herbivores.

5. Conclusion

Pre-hardening seed treatments of cowpea with salicylic acids was found to enhance tolerance capacity of cowpea to pod sucking bug, *C. tomentosicollis*. The different pre-hardened cowpea varieties screened showed variable response to the bug attack. Pre-treatment effects with hormone reveal that 10ppm Salicylic acid (SA) treatments improve tolerant capacity of cowpea than the lower (5ppm) concentrations and the controls. Of the four Cowpea varieties screened, IT97K-1069-6 and IT89KD-288 of 10ppm SA treatments were found tolerant owing to their low susceptibility indices and damage resulting to prolonged developmental time of nymphal bugs with few progeny emergences while Dan'ila and IT98K-205-8 treated from same concentration were however susceptible. Phytochemical screening of the treatments using GC-MS analysis showed appreciable concentrations of cumene, eugenol and sesquiterpenes in the tolerant varieties. These relations should be explored extensively toward sustainable plant protection.

6. Acknowledgement

The authors acknowledge the financial support of Tertiary Education Fund for partly funding this research work and facility support from Department of Biological Sciences, Bayero University and National Research Institute for Chemical Technology (NARICT), Zaria.

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