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Evaluation of Salicylic acid Pre-Hardening Treatments of Cowpea for Resistance against the Flea Beetle, *Podagricafuscicornis* Linn. (Coleoptera: Chrysomelidae)

Audi, A. H.[1], Mukhtar, F. B.[2]

[1]Department of Biological Sciences, Faculty of Life Sciences, Bayero University, PMB. 3011, Kano Nigeria
[2]Department of Plant Biology, Faculty of Life Sciences, Bayero University, PMB. 3011, Kano Nigeria
Corresponding author: audigenesis@yahoo.com

Abstract— *The experiment was conducted 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 bugs 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 bug attack. IT97K-1069-6 and IT89KD-288 recorded lower indices of susceptibility and leaf damage to flea beetle attack, delayed development time of larvae with few progeny emergence. Pre-treatment effects also varied significantly (P<0.001) with higher concentrations (10ppm) of Salicylic acid (SA) being more effective in reducing leaf damage 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 10ppm of SA were found tolerant owing to their low susceptibility indices (7.09 and 9.09) and percentage leaf damages (18.58% and 25.93% respectively while Dan'ila and IT98K-205-8 treated from the same concentrations were however susceptible. Phytochemical analysis of treatments showed high concentrations of eugenol and sesquiterpenes in the tolerant varieties. These relations should be explored extensively toward sustainable plant protection.*

Keywords— Cowpea, Flea beetle, Management, Phytochemicals, Salicylic acid.

I. INTRODUCTION

Cowpea leaf damage by flea beetles is increasingly becoming a threat to photosynthesis and production of essential nutrients by the plant at early stage of establishment.Although the pests has been classified as a minor pests of legumes, it is becoming one of the major cowpea leaves pest which re-currently appear at early planting stage causing characteristic pores on cowpea leaves in the absence of its other host. This significantly retards development of cowpea which may decrease its recovery potential against other leave insect pests such as Aphids. Management approach using Chemical insecticides have posed detrimental effects to humans and the environment, and are also not affordable to majority of peasant farmers (Alebeck, 1996). Alternative approach towards efficient and cost effective means of production of cowpea is very desirable. The development and use of resistance legume varieties offer a simple, cheap and attractive approach to the reduction of pest damage (Ofuya, 2001). Host plant resistance is the easiest, most economical and effective way of managing insect pests and diseases. It requires low technological inputs, create low environmental hazard and is generally compatible with other methods of pest control. Inducible defenses are reported to improve plant fitness and may be more durable, than constitutive defense mechanisms (Agrawal, 1998; Agrawal, 1999).

1.1. The Flea Beetle, *Podagricafuscicornis*

Flea-beetles, *Podagrica spp*. are classified into family leaf beetles (Chrysomelidae) and subfamily Alticinae(Aslan *et al*., 1996) and occur in all vegetation habitats. *P. fuscicornis*belongs among oligophagus herbivores and it induces damage on plants from genera *Althaea, Malva*and *Lavatera*(Cmoluch, 1988; Rotrekl, 1996; Brelih*et al*., 2003).It can cause damage also on plants from families Lamiaceae, Urticaceae and leguminosae(Petitpierre, 1985). Flea beetles have been described as small bugs with a size from 1.5 to 4 mm. They can jump due to the enlarged backside organ (hind legs). They also have the ability to fly. When plant is heavily attacked by flea beetles, small round holes (up to 1 mm) caused by an individual flea beetles feeding may coalesce into larger areas of damage (Gruev and Doberl, 1997). Adult beetles of *P.fuscicornis*are 3.0 to 6.0 mm in size. Head and neck shield are coloured red. Front wings (sheath-wings) are dark blue to blue green and rarely are detected metal coloured. Sheath wings contain hollows which are deep, very abundant and spread scarced. Hollows on neck shield are even more compacted. Legs are characteristically yellowish to bright brownish, and upper labium is also yellow (Hubble, 2010).

1.1.1. Life cycle of Cowpea Flea Beetle

Flea beetles are insects with complete metamorphosis, adult females lay eggs, which hatch into larvae of various shapes. After feeding and moults several times, larvae mature, pupate and later emerge as adult beetles; the length of time it takes to complete the life cycle varies greatly from species to species and is also dependent on weather and other environmental conditions (RakCizej*et al*., 2001). Cmoluch (1988) reported that the female lays its small yellow eggs into the soil at the base of the host plant. The egg stage took 7-13 days to hatch and larvae hatch after 7–11 days and feed for a period of 11-28days on the rootlets. Pupation takes place in the soil and the adults emerge after 10-17 days from the pupa.The adult remains after rain on the host plant for as long as they can find suitable food (Breli*het al*., 2003).They always prefers young growth. Physical and chemical factors of plant defence many times have influence on the survival ability of larvae. The latter can also be affected by the appearance of natural enemies andunfavourable weather conditions (RakCizej*et al*., 2001). Larvae molt three to four times and then pupate. After one to two weeks, the adults emerge. The Chrysomelid has only one generation per year (Cmoluch, 1988). Adults overwinter usually in the soil under the grass sward, where often enough organic matter or nearby host plant (RakCizej*et al*., 2001). Very known members of genus *Podagrica*cause most damage on plants from family Malvaceae.

Numerous control measures, including physical, chemical, biological and cultural have been employed by farmers to combat many devastating pests in cowpea seed production and storage. However, the continuous uses of insecticides at increasing dosage in order to compensate its ineffectiveness due to insect resistance are also potential threats to aquatic fauna, environmental and human health. Alternative approach towards efficient and cost effective means of production of cowpea is very desirable. Host plant resistance is the easiest, most economical and effective way of managing insect pests and diseases. Inducible defenses are reported to improve plant fitness and may be more durable, than constitutive defense mechanisms (Agrawal, 1999). Previous attempt to use plant growth hormones such as salicylic acid (Stout *et al.*, 1999; Alvarez, 2000; Walling, 2000; Aviv *et al.*, 2002; Brodersen*et al*., 2002) to induce resistance in some plants have been reported. However, little was found on how Salicylic acid (SA) affects resistance of cowpea to insect pests. While most research uses plant growth hormones exogenously as surface spray to control insect pests, this research investigates to test the potential of salicylic acid (SA) to induce resistance against insect pests using pre-sowing hardening treatments. Salicylic acid have been classified as safe and environmentally friendly, practically non-toxic and biodegradable with low potential for bioaccumulation (EU regulation on chemicals: 1272/2008 (EEC, 2008). The aim of the research is to assess the physiological effect of salicylic acid in Cowpea against the Leaf feeding beetle, *Podagricafuscicornis* (L)

II. 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 and10ppm 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,

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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 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 stands 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. Insect Collection and Method of Infestation

Five pairs of adult males and females of freshly mated flea beetle, *Podagricafuscicornis* were collected from the exposed field while mating 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 separate from copulation or take flight when disturbed. Infestation was made during the late July (4-5 weeks after planting) at pre-booting stage. 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 larval beetles 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 (Underwood *et al.,* 2002).

2.3.1. Effect of Pre-hardening treatments on Progeny emergence and Developmental time of flea beetle, *Podagricafuscicornis*

To determine preference for insects' fecundity, on the different cowpea varieties grown in the various field cages, four-weeks old seedlings were infested with five-pairs adult males and females flea beetles, *P.fuscicornis*. The insects were allowed to mate and oviposit for one week after which they were removed from each plant. Clutches of yellowish eggs and egg masses were observed on surface soil at the undergrowth. These were maintained undisturbed for larval development but observed regularly until adult beetle emerged. Insects were counted and discard 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 *P.fusicornis*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 Leaf Damage of *P.fuscicornis*

A similar set up was maintained unperturbed until all adults insects emerged but allowed to feed over 2 weeks periods beyond which few adults survived. Visual leaf damage caused by larval beetles during period of exposure (2 weeks) was determined based on scale rating by Panda and Kush (1995), Jackai and Singh (1988).Percentage leaf damage was calculated using the formular;

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

2.3.3. Effect of Pre-hardening treatments on leaves Susceptibility to *P. fuscicornis*

Based on the cumulative emergence of F_1 insect progeny and mean development time, the susceptibility indices of the different cowpea cultivars to flea beetle attack was evaluated (Dobie, 1974) using the following formula;

$$SI = \frac{\text{Loge}Y \times 100}{D}$$

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 % proportion of each

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component was determined 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 determine by comparing spectrum of the known component with that of the known components stored in the NIST library (Valls*et 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) version10.3DE, Rothamsted Experimental Station.

III. RESULTS

The effect of the different hormones (5 ppm and 10 ppm of SA) on infestation of flea beetle is shown in Table 1. There was significantly less progeny emergence in the Salicylic acid pre-hardened cowpea seeds with 5ppm SA showing considerable reduction in the number of progeny emergence (18.50) than with other treatments. But progeny emergence was significantly higher in both the distilled water treatment and the control (49.42 and 55.08) respectively (Table 1).

Table.1: Effect of Salicylic acid (SA) Pre-hardening Treatments of Cowpea on Developmental time (days) of Podagricafuscicornis

Hormone treatments	**Cowpea varieties**				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	15.33	20.00	15.67	23.00	18.50
10ppmSA	11.67	17.00	21.00	27.33	19.25
DIST. H2O	47.33	49.33	51.00	50.00	49.42
CONROL	53.67	54.00	65.33	47.33	55.08
MEAN	32.00	35.08	38.25	36.92	35.56
LSD5%	11.96	10.69	13.22	7.612	10.76

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

Developmental time of the beetle 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<0.001). Developmental was observed to be significantly longer (P<0.001) in all the hormone treatments when compared with the controls (Table 2). Larval development time was however longer in the 10ppmSAtreatments (34.17days) than the lower concentration (5ppmSA).

Table.1: Effect of Salicylic acid (SA) Pre-hardening Treatments on Progeny emergence of Podagricafuscicornis

Hormone treatments	Cowpea varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	30.67	35.00	29.67	30.00	31.33
10ppmSA	37.00	38.00	32.00	29.67	34.17
DIST. H2O	29.67	28.67	27.33	26.00	27.92
CONROL	25.67	30.00	30.00	26.33	28.00
MEAN	30.75	32.92	29.75	28.00	30.36
LSD5%	2.602	2.411	1.061	1.179	1.663

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

Table 3 shows the response of the different pre-hardened cowpea varieties to attack by Flea Beetle, *Podagricafuscicornis*. The tolerance capacity interms of susceptibility index and leaf 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<0.001). The susceptibility index was remarkably lower in the entire hormone treated cowpea seeds compared with the distilled water treatment and the control. Treatment with 10ppmSA resulted in significantly (P<0.001) lower susceptibility indices (9.88) to leaf feeding beetles when compared with 5ppmSA (10.18) and the controls. Varietal response shows that IT97K-1069-6 and IT89KD-288 were tolerant (9.45 and 10.22 respectively) compared with IT98K-205-8 and Dan'ila with mean response of 11.01 and 12.52 respectively.

Table.3: Effect of Salicylic acid (SA) Pre-hardening Treatments on Cowpea Leaves Susceptibility (SI) to Podagricafuscicornis

Hormone treatments	Cowpea varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	9.12	9.23	10.02	11.15	9.88
10ppmSA	7.09	9.09	12.56	11.99	10.18
DIST. H2O	10.60	10.78	10.37	13.11	11.21
CONROL	10.98	11.76	11.09	13.84	11.92
MEAN	9.45	10.22	11.01	12.52	10.85
LSD5%	0.978	0.711	0.623	0.659	0.521

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

Percentage leaf damage was significantly (P<0.001) lower in the hormone treated seeds compared with the distilled water treatment and the control in which the percentage damage was greater than 60%. Comparison of the hormone treatments indicated that the least Percentage leaf damage was obtained in cowpea seeds treated with 10ppmSA (32.55%) than the lower concentration. Varietal response to treatments shows that IT97K-1069-6 and IT89KD-288 recorded significantly (P<0.001) lower percentage leaf damages (42.42% and 43.42% respectively) than the other varieties in which more than 50% leaf damages were observed (Table 4).

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 (Appendix 1).

Table.4: Effect of Salicylic acid (SA) Pre-hardening Treatments of on Cowpea leavesDamage(%) byP. fuscicornis

Hormone treatments	Cowpea varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	27.42	27.77	57.02	54.65	41.72
10ppmSA	18.58	25.93	38.49	47.19	32.55
DIST. H2O	67.73	62.74	61.15	77.36	67.24
CONROL	55.89	57.22	67.13	79.05	64.82
MEAN	42.42	43.42	55.95	64.56	51.58
LSD5%	12.87	10.69	6.852	8.907	9.498

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not Significantly different, P<0.001.

IV. DISCUSSIONS

Pre-hardening treatments of cowpea in various concentrations of salicylic acids resulted in varied responses to flea beetle attack. Cowpeas treated with 10 ppm SA were more tolerant to flea beetle attack than at lower concentrations (5 ppm). The population of the beetles was considerably high in control of IT98K-205-8 and Dan'ila due to high emergence but was low in the less susceptible varieties. The development time of the beetle was longer in the 10 ppm SA treatments of IT97K-1069-6 and IT89KD-288 than the other treatments and controls in which the shortest development time was observed in Danila variety. This delayed developmental time and fewer progeny emergence are consequent of inducible defense elicited by salicylic acid treatment which may have effect on the nutritive value of the diet or indirectly the effect of antifeedants detected in the leaves, mainly tannins and phenolic derivatives (2-hydroxyethoxyethyl and Quinolines respectively) in the less damage varieties. This may be related with the reports of Barbehenn and Peter (2011) who revealed that, ingested tannins reduce the digestibility of the proteins to insect herbivores thereby decreasing the nutritive value of plants and systemically induced neighboring leaves of the damaged plants. A similar report have also show that higher production of salicylates compounds may considerably affect the available nutrients or could reduce protein digestibility in insects (Barbehenn and Peter, 2011).

The susceptibility index (SI) of also varied significantly with varietal type and hormone- treatments. Pre-hardening treatments of IT97K-1069-6 and IT89KD-288 in 10 ppm SA confer resistance to the flea beetles attack than with other treatments in which Dan'ila variety was more prone to infestation and damage by the beetles owing to their high susceptibility index and damage levels. Varieties with low susceptibility indices were less damaged and are therefore tolerant to the flea beetle attack. Characteristic leaves windowing which extended towards the margin were irregularly made to varying degrees. Crops with such damage show reduce flowering and pod productions due to impaired photosynthesis and movement of nutrient across the leaves palisades. Susceptibility index is a measure of crop resistance; a high susceptibility index implied that more progeny developed from a variety over a shorter time. The low susceptibility of IT97K-1069-6 and IT89KD-288 treatments could be attributed to higher levels of glucosinolates (Butylhydrogenpthalate) and phenolics (Octadecadienol) detected in the varieties which may have adversely affected the utilization of nutrients by the leaves feeding beetle (Table 3). This may be related to the findings of Duffey and Stout (1996) and Bhonwong*et al.*, (2009) who reported that, Quinones formed by oxidation of phenols bind covalently to leaf proteins, and inhibit the protein digestion to insect herbivores. Reports in the same vein have similarly show that, changes in defensive constituents of a plant on account of insect attack develop unpredictability in the plant environment for insect herbivores, which in turn affects the fitness and behaviour of the herbivores (Miranda *et al.*, 2007).

V. CONCLUSION

Pre-hardening treatments of cowpea with Salicylic acid significantly influenced various regulatory responses to leaf feeding beetle *Podagricafuscicornis.*Cowpea varieties IT97K-1069-6 and IT89KD-288 recorded low indices of susceptibility and leaf damage to flea beetle attack, delayed development time of larvae with few progeny emergence. Pre-hardening treatments with higher concentrations (10ppm) of Salicylic acid (SA) was effective in reducing leaf damage than the lower (5ppm) concentrations and the controls. Of the four Cowpea varieties screened, IT97K-1069-6 and IT89KD-288 pre-hardened with 10ppm SA were found tolerant to flea beetle attack owing to their low susceptibility indices and damage levels while Dan'ila and IT98K-205-8 of the same treatments were however susceptible.

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Appendix 1.

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

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	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	Pseudocumol	4.95	6.67	Octadecadienol
	19.64	7.49	2-hydroxyethoxyethyl (Phenolic)	3.51	6.83	Ethylbenzol	3.51	9.03	Pentadecanecarboxylic acid, ethylbenzol	4.95	6.15	Ethylbenzol
10ppm SA	22.36	24.71	Phytol (Diterpene),	23.78	28.28	n-Hexadecanoic acid	22.16	7.23	o-Methyltoluene	3.58	4.09	Cyclohexane
	22.36	13.68	2-hydroxyethoxyethyl (Eugenol)	23.78	13.74	Pentadecanecarboxylic acid, ethylbenzol	22.16	8.09	Pentadecanecarboxylic acid	3.51	6.50	Ethylbenzol
	22.36	15.55	Quinolines (Tannin) Iodomethylbenzoic.	23.78	14.88	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