

# Effects of Plant Derived Salicylic Acid on Growth and Development of Moringa Budworm Larva, *Noorda moringae* (Lepidoptera: Crambidae)

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## Abstract

The experimental trials were carried out to evaluate the impact of plant-derived salicylic acid (SA) treatment on the development of moringa budworm. The SA obtained from plant sources were form into three different concentrations (5mg/l, 10mg/l, and 20mg/l) by serial dilution. Moringa plants were treated by spraying with varying doses of SA hormones at week intervals. The treated plants were blocked in screened cages in a completely randomized blocked pattern. Five pairs of freshly emerged *Noorda moringae* were maintained in the various blocks for mating and oviposition, after which all adult insects were removed thereafter. The treated moringa plants exhibited different responses to budworm development. There was a significant ( $P < 0.001$ ) effect among hormone concentrations and spray regimes, with 20mg/l salicylic acid (SA) showing greater influence on various developmental stages of larval *N. moringae* compared to lower concentrations (10 & 5mg/l SA) and the control (Distilled H<sub>2</sub>O). The higher levels of SA at median spray regime (2 weeks after spray) hindered larval development more, as evidenced by low growth rate (RGR) and Larval Fitness Indices (LFI) respectively. Phytochemical analysis of the extracts revealed notable concentrations of 9-octadecanoic acid methyl ester, Methyl tetradecanoate, Methyl 4-hydroxy butanoate, and Hexadecanoic acid methyl ester, which displayed toxic effects on the developing larvae.

**Keywords:** Control, *Moringa oleifera*, *Noorda moringae*, phytochemical analysis, Salicylic acid

## INTRODUCTION

The Biology of Moringa budworm, *Norda moringae* which constitute its growth and developmental cycle are the structural frame work to understanding its behavior. The interaction with its associated host forms the integral part of its ecology. The cryptic nocturnal feeding behavior of its developing larvae would be the key target in holistic control. Manipulating the plant host by altering the chemical or nutritive composition of its plant host

could strongly impede larval growth and development as well as reproductive capacity of the insect.

It is well established that, *Moringa oleifera* has a great potential to serve as a high-value food crop, medicinal products, as well as fodder for animals, particularly in developing countries (Shahzad *et al.*, 2013). The leaves present a very high content of fiber, antioxidant and bioactive compounds (Leone *et al.*, 2016; Jung *et al.*, 2018). Reports have shown that production of this important plant had continuously been hampered by infestations from many insects, among which *Norda moringae* was implicated to causing high damages to the flower bud of *Moringa oleifera*.

Many attempts have been made to protect the plant against insect pest using insecticides which due to its adverse effects on ecosystems calls for alternative and more sustainable approach. It was reported that, salicylic acid has potentials of altering physiological activities of insects (Elhamahmy *et al.*, 2016). Exogenous application of SA has been used to induce adaptive responses in plants to both abiotic and biotic stress (Rana *et al.*, 2016; Lortzing *et al.*, 2019).

In addition, a report by several authors: Eulgem (2014), Shi *et al.* (2016), Yang *et al.* (2016) Erb and Reymond (2019) and Aerts *et al.* (2021) highlighted that, the application of SA externally, either through spraying or seed treatment, can imitate responses to both abiotic and biotic stress. This includes defense mechanisms activated by plants, pathogenic microorganisms and insects.

Exploring this relation will align to the current trend and approaches of integrated control of insect pests. The research had therefore resort to test the potentials of salicylic acid extracted from plants to alter growth and development of *Norda moringae* with a view to its effective management.

## MATERIALS AND METHOD

### Study Area

The field trials were carried out at Satame, Makoda Local Government area. Kano State (12°18'20"N, 8°36'30"E / 12°16'0"N, 8°27'50"E) from October 2020 to November 2021. The average annual rainfall ranged from 631mm to 1250mm, with mean at 20°C to 38°C. The relative humidity during this period was recorded between 64 and 90mmHg, according to NIMET (2021).

### Formulation and Assignment of Treatments

The separations of various air dried organic compounds extracted from tomato plants were passed through columns separation following similar method used by Vivek *et al.* (2016). The fractions containing salicylates isolates were subjected to serial dilution to produce the various concentrations. The plants were established into four replicates in screen cages on a complete randomized block design, with the level of SA treatments representing blocks and spray regimes as treatment levels respectively and blocks sprayed with distilled water (Dist. H<sub>2</sub>O) represents the control groups (Elhamahmy *et al.*, 2016).

### Method of Infesting and Insects Collection

Each of the blocks described above were infested with 10 larvae of *N. moringae*. The newly hatched adult moths of the budworm were separated and released in pairs into a separate,

fresh experimental setup to determine growth and reproductive parameters such as oviposition, larval emergence, and larval weight.

#### **Determination of Developmental Parameters of immature Stages of *Norda moringae***

To assess fecundity per female moths, the egg /clusters were observed on flower bud using a hand ocular lens. These were counted daily until ovipositing moths died. The percentage of egg hatchability, were determined by collecting egg masses from each replicate. Eggs were monitored daily until first instar larva emerged and the percentages of hatched eggs were determined as follows:

$$\text{Egg hatchability (\%)} = \frac{\text{No. of larvae emerged from each egg Clusters}}{\text{Total No. of eggs per Cluster}}$$

The total number of larvae that emerged through the developmental instar period was determined by the count for each treatment. Larval developmental time was determined as the period of egg hatching to the termination of growth phase, on which larvae entered the wandering stage (Davidowitz *et al.*, 2003).

#### **Determination of Larval weight, Growth rate and Fitness Index of *Norda moringae***

Full grown larvae collected from respective treatments were weighed from the 3<sup>rd</sup> larval instar after every 24 hours of feeding, using a Mettler AE50 analytical balance. The mean weight gained by larvae per each treatment were estimated as weight difference from the initial instar to the last (5th) instar period prior to entry into the wandering stage (Davidowitz *et al.*, 2003). The larval growth phase of *Norda moringae* was monitored until pupation and the relative growth rate was estimated following the procedure as described by Pavana *et al.* (2023). The Larval Fitness Index (LFI) of *Norda moringae* derived from earlier work of (Itoyama *et al.*, 1999) was estimated in the present work, as the proportion of emerged larvae and its weight equivalent to the sum of larval period and its weight using the following Equation:

$$\text{Larval Fitness Index (LFI)} = \frac{\text{Le(Lw)}}{\text{Lw} + \text{LP}}$$

Where: LP = larval period, Le = percentage Larval Emergence and Lw = Larval weight.

#### **Statistical Analysis**

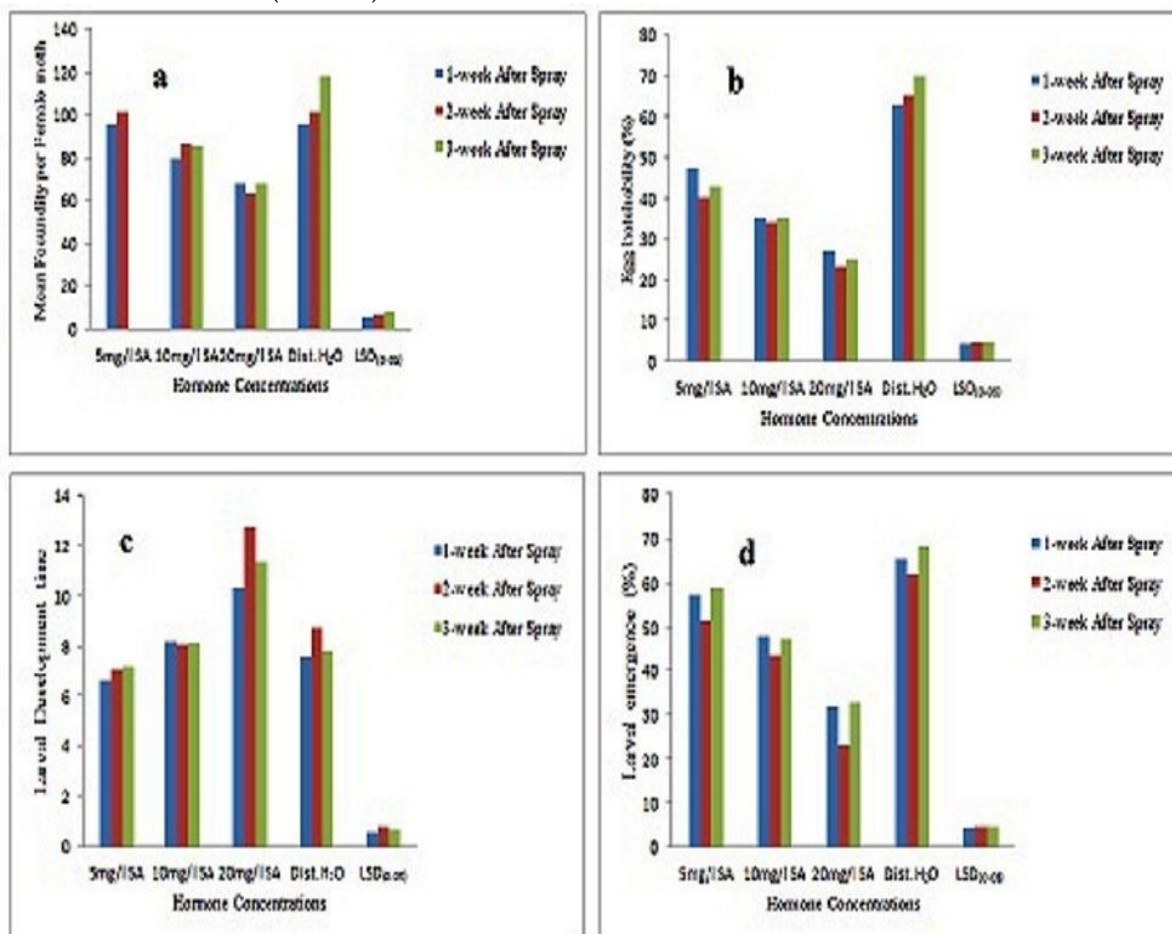
A randomized block design was utilized, with various concentrations of SA designated as blocks and the spray regime representing treatment levels. All percentage were arcsine transformed and count data to square root transformation. The data were subsequently subjected to two way Analysis of variance (ANOVA) in randomized block design using SPSS software version 20

## **RESULTS**

The results of this research showed significant variation in the developmental parameters of *Norda moringae* larvae among the different SA-treated moringa plants and spray regimes. The result of Oviposition, egg hatchability and larval emergence was presented in Figures 1a, b & c respectively. The immature stages mentioned above were significantly affected by higher concentrations (20mg/l SA) and median spray regimes (2-WAS) compared to other treatments. A notable variation ( $P < 0.05$ ) was observed in fecundity, egg hatchability, and larval emergence across different treatments.

The Larval growth, weight and development time to various instars also varied significantly ( $P < 0.05$ ) between treatments (Fig 1d). The budworm's development was notably prolonged at the higher treatment (20mg/l SA), leading to significant larval weight loss and lower growth rate owing to their lower growth rate (0.5411) and fitness index value (0.0060) respectively

(Tables 1, 2 &3). This developmental failure was also evident by the higher larval mortality in the same treatments (Table 4).



Means with variations lower than the LSD values at a 5% significance level are considered not statistically significant ( $P < 0.05$ ).

Fig 1. Influence of Salicylic Acid (SA) on Developmental Parameters of immature Stages of *Norda moringae*: a. Mean fecundity per female moth, b. Percentage egg hatchability, c. larval developmental time & d. Percentage larval emergence

Table 1: Influence of Salicylic Acid (SA) on the weight of *Norda moringae* larvae.

Hormone Conc.	No. of Moringa Stands	Weight Before Spray	Spray Regimes		
			1-week After Spray	2-week After Spray	3-week After Spray
5mg/1 SA	4	0.00531	0.00465	0.00422	0.00477
10mg/1 SA	4	0.00531	0.00461	0.00422	0.00475
20mg/1 SA	4	0.00531	0.00457	0.00419	0.00463
Dist. H <sub>2</sub> O	4	0.00531	0.00465	0.00477	0.00477
LSD5%			3.438	3.388	3.502

Means with variations lower than the LSD values at a 5% significance level are considered not statistically significant ( $P < 0.05$ ).

**Table 2: Influence of Salicylic Acid (SA) on Larval Growth Rate (RGR) of Moringa Budworm *Norda moringae***

Hormone Conc.	No. of Moringa Stands	Spray Regimes			Mean (RGR)
		1-week After Spray	2-week After Spray	3-week After Spray	
5mg/l SA	4	0.6461	0.4194	0.7738	0.6131
10mg/l SA	4	0.6133	0.4194	0.7487	0.5938
20mg/l SA	4	0.5840	0.4101	0.6292	0.5411
Dist. H <sub>2</sub> O	4	0.6461	0.7738	0.7738	0.7312
LSD5%		0.1925	0.2013	0.1856	0.1929

Means with variations lower than the LSD values at a 5% significance level are considered not statistically significant (P<0.05).

**Table 3: Influence of Salicylic Acid (SA) on Larval Fitness Index (LFI) of Moringa Budworm, *Norda moringae***

Hormone Conc.	No. of Moringa Stands	Spray Regimes			Mean (LFI)
		1-week After Spray	2-week After Spray	3-week After Spray	
5mg/l SA	4	0.0201	0.0037	0.0038	0.0092
10mg/l SA	4	0.0197	0.0037	0.0039	0.0091
20mg/l SA	4	0.0120	0.0029	0.0032	0.0060
Dist. H <sub>2</sub> O	4	0.0302	0.0040	0.0040	0.0128
LSD5%		0.22837	0.22929	0.22928	0.22898

Means with variations lower than the LSD values at a 5% significance level are considered not statistically significant (P<0.05).

**Table 4: Influence of Salicylic Acid (SA) on Larval Mortality (%) of *Norda moringae***

Hormone Conc.	No. of Moringa Stands	Spray Regimes		
		1-week After Spray	2-week After Spray	3-week After Spray
5mg/l SA	4	36.35	38.31	35.31
10mg/l SA	4	50.95	50.11	55.11
20mg/l SA	4	73.99	73.15	78.15
Dist. H <sub>2</sub> O	4	0.000	2.150	3.030
LSD5%		5.8654	5.7689	6.1525

Means with variations lower than the LSD values at a 5% significance level are considered not statistically significant (P<0.05).

## DISCUSSION

The findings of the study revealed notable variations in the developmental parameters of *Norda moringae* among the different moringa plants treated with salicylic acids (SA) and subjected to different spray regimes. The higher levels of SA treatments exhibited significant obstacles to the oviposition and egg hatchability of *Norda moringae* compared to the lower concentrations and the control group. This finding can be related to the work of Groux *et al.* (2014) who reported that exogenous or constitutive application of SA at high levels, deterred oviposition by *P. brassicae* in willows. Another related study documented a decrease in both fecundity and egg hatchability in *S. frugiperda* when exposed to methanol extracts derived from medicinal plants (Freitas *et al.*, 2014).

The emergence and development of larvae into instars followed a similar pattern. *Moringa oleifera* seedlings treated at higher level of salicylic acids (SA) reduced larval emergence from deposited eggs and growth of the developing larval budworm, *Norda moringae*. This effect can

be attributed to the production of some toxic metabolites that reduced egg viability and subsequent production of defective larvae due to low metabolic activity.

The developing larvae had also experienced significant weight loss and decreased fitness at higher treatment levels (20mg/l), two weeks after the spray. The treatment may activate production of some defensive metabolites. These compounds might hinder nutrient availability or have a toxic effect, resulting in retarded larval growth and significant weight loss. This aligned to the findings of Damodaram *et al.* (2015) who observed that application of salicylic acid increased the level of toxic compounds such as polyphenol oxidase and hydrogen peroxide which potentially decrease the growth of larvae or nymphs. Report of Nijhout and Callier (2015) also showed a decrease in the specific growth rate as body size increases, suggesting that larger portion of the larva may consists tissues with lower metabolic activities.

Higher concentration of 20mg/l SA and the median spray regime of 2-WAS significantly delayed the development of different larval stages, resulting in a high larval mortality rate due to failure to pupate or survive to pupation. The reduced fitness of the larvae led to a decrease in food utilization capacity, causing poor digestion and a lower relative growth rate. Related findings also suggested that plant-based products may affect Phago-stimulation receptors in the mouth, potentially reducing appetite or deterring feeding behavior (Ali *et al.*, 2017).

The reproductive capacity and larval competitiveness also decreased remarkably, as less food is being utilized, resulting in stunted larval growth and increased developmental failure. The plant might have benefitted positively with enhanced production of secondary metabolite that increase its tolerance. This is evidenced by the rise in larval mortality. These aligned with the study conducted by Aerts *et al.* (2021) who reported involvement of SA in plant resistance to herbivorous insects. A Similar report demonstrated the toxic effects of Phenolic compounds on larval development of certain insects (Movva and Pathipati, 2017).

## CONCLUSION

The findings revealed that larval growth and development of *N. moringae* was strongly impaired at higher level of SA treatment. The results showed significant variation in the developmental parameters of *Norda moringae* larvae across various treatments. Oviposition, egg hatchability and larval emergence were significantly affected by higher concentrations (20mg/l SA) and median spray regimes (2-WAS) compared to other treatments. The budworm's development was notably prolonged at the higher treatment, leading to significant larval weight loss and lower growth rate owing to their lower growth rate (0.5411) and fitness index value (0.0060) respectively. This developmental failure was also evident by the higher larval mortality in the same treatments. These relations should be utilized to induce tolerance to *Moringa oleifera* against the larval budworm.

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