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**Utilizing Plant-Derived Salicylic Acid to Inhibit Leafminer Moth
(Noorda blitealis Walker) Infestation on *Moringa Oleifera***

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ABSTRACT

The experimental trial was conducted at Sakab Farm Limited Satame, Makoda Local Government, Kano State (12°18'20''N, 8°36'30''E / 12°16'0''N, 8°27'50''E), from October, 2021 to November, 2022. The experiments were established in various field cages in a split plot design with various levels of Salicylic acid (SA) treatments representing the main plots and the spray regimes: 1 weeks after spray (1-WAS), 2 weeks after spray (2-WAS) and 3 weeks after spray (3-WAS), as subplots. Different Concentrations of SA (5mg/l, 10mg/l and 20mg/l) were extracted from plant sources and formed by serial dilution. Ten freshly emerged larvae of *Noorda blitealis* collected from infested fields were introduced into each of the treatment group containing the treated stands of drumstick. The set up was undisturbed but observed at regular intervals. The results shows that, mean percentage leaves damage varied significantly ($P<0.001$) among different concentrations of SA and the spray regimes. Larval development time was significantly ($P<0.05$) prolonged in the higher concentration (20mg/l. SA) and median spray regime (2-WAS). The mean percentage leaves damage and progeny emergence of the moths were considerably low at the same treatment combinations. Moringa plants treated with higher concentrations of SA were also less infested and apparently more tolerant than the other treatments owing to their lower index of susceptibilities. This facility should be incorporated into the integrated control for effective management of Insect pest.

Keywords: *Moringa oleifera*, *Noorda blitealis*, Infestation, Salicylic acid

1.0. INTRODUCTION

Moringa has multipurpose use, well adapted and significant economic importance, as it has vital nutritional, industrial, and medicinal applications (Dawit *et al.*, 2016). *Moringa oleifera* has the great potential to become one of the most economically important tree crops for the tropics and sub-tropics. However, *Moringa* production is hampered by several diseases and insect pests (Tamraj and Gupta, 2020). Among its major insects pest is the moringa leaf caterpillar, *Noorda blitealis* Walker 1859 (Halder *et al.*, 2014; Math *et al.*, 2014; Litsinger, 2014; Joshi *et al.*, 2016; Sharjana and Mikunthan, 2018; Kabré, 2021). It can cause 100% defoliation and thus poses a threat to moringa production (Brunda *et al.* 2015). *Noorda blitealis* is a lepidopteran belonging to the Family Crambidae whose larva or caterpillar is the damaging stage on the moringa tree. Due to the significant expansion of moringa cultivation in the world, the pest is continuously expanding its area of distribution in recent years. It is reported as a pest of moringa in

Asia where it originates and in several other tropical regions (Joshi *et al.*, 2016; Thumar *et al.*, 2017).

The use of various insecticides from time to time has created a number of risks, like genetic resistance in pest insects, contamination of food products with toxic residues, increased cost of application, handling hazards, ecological disorders and high mammalian toxicity (Hladik *et al.*, 2018; Zhen *et al.*, 2019). It is highly desired to look for alternative approaches to productive and affordable moringa production. Salicylic acid a plant hormone was reported to provide immunity to the plant against pathogens and insect pests (Elhamahmy *et al.*, 2016). Induction of SA pathway with exogenous elicitors was successfully conducted using a variety of compounds including SA proper, MeSA, and Benzothiadiazole (BTH, benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester, a synthetic analog of SA), and S-methyl methionine salicylate, among others (Bektaş *et al.*, 2015).

Exogenous applications of SA, either by direct injection, spraying and pre-hardening treatment, have been reported to cause multitude effects on the morphology and physiology of plants. It acts as the signaling molecule for triggering system acquired resistance (Pancheva *et al.*, 1996; Durrant and Dong 2004; Audi and Mukhtar, 2019).

The research work intended to utilize these relations to induce tolerance on *Moringa oleiferae* against the leaf caterpillar *Norda blitealis*. The concept is in tandem with the integrated insect pest management approach and has been found to be environmentally friendly and cost effective methods (Audi and Mukhtar, 2019). The overall aim is to assess the effect of plant derived salicylic acid in suppression of infestation of *Moringa* leaf caterpillar, *Norda blitealis*. The specific objectives are;

- i. To determine developmental time and progeny emergence of *Norda blitealis* on SA treated *Moringa* plants
- ii. To assess the susceptibility Indices and leaves damage of the treated *Moringa* plants to attack by *Norda blitealis*

2.0. MATERIALS AND METHOD

2.1. Study Area

Sakab Farm Limited Satame, Makoda Local Government, Kano State (12°18'20"N, 8°36'30"E / 12°16'0"N, 8°27'50"E) was the site of the experimental field trials, which ran from October 2020 to November 2021 (Department of Geography, BUK, Cartography Lab). The average annual temperature ranged from 20 to 38°C, the average annual rainfall was between 631 and 1250 mm, and the relative humidity was between 64 and 90 mmHg (Nimet, 2021)

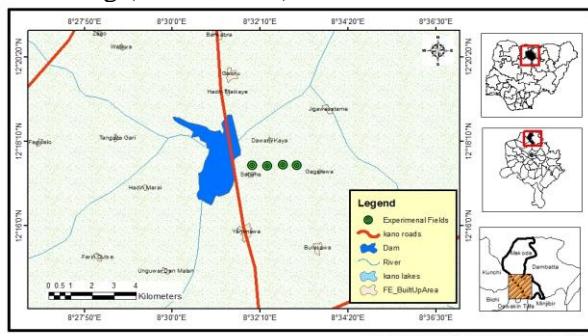


Figure 1: Map of Experimental Site (Sakab Farm Limited Satame Makoda Local Gov.), Kano State. Source (Cartography Lab BUK, 2022)

2.2. Seedling Establishment

Moringae oleifera seeds were identified and collected at Kano State Agricultural and Rural Development Authority (KNARDA). The seeds were sown in various screen cages 4-seeds were placed per hole and were thinned to 2 seedlings per stand for screening against *Moringa oleifera* 2.2 during the month of October 2020 to October 2021.

2.3. Formulation of Treatments

The Phenolic compounds were extracted from *Solanum lycopersicon* powder using column chromatography based on method of Bektas *et al.* (2015). SA were isolated by Liquid chromatography mass spectroscopy. The salicylic acid solution were prepared in the laboratory by dissolving 1gram of the extract in 1ml of 75% ethanol for dilution in distilled water to make the stock solution. These were subsequently diluted to various concentrations (5mg/l, 10mg/l and 20mg/l) following the methods of Darra *et al.* (1973) and Audi and Mukhtar (2019).

2.4. Exogenous foliar Spray

During the twilight hours, when the air is calm, the 12-week-old *Moringa* seedlings were sprayed with several doses of salicylic acid solution using a backpack sprayer. Two repetitions of each week were made of these. This will enable adequate treatment absorption throughout the vascular system of the plant. An infestation of treated plants was prevented in a 3–4 m screened house (Audi and Mukhtar, 2019).

2.5. The method of infesting and collecting insects

Freshly emerged larvae of the moths were collected from the infected field at early morning hours when the larvae are relatively less active. The plants were infested with 10 larvae of *N. blitealis* at different induction time (1-week after spray, 2-weeks and 3-weeks after spray) in various screen cages. These prevent entrance by other insects and ensure accurate assessment of infestation due to the leaf caterpillar *Norda blitealis* (Underwood *et al.*, 2002; Audi and Mukhtar, 2019).

2.6. Estimating the Developmental Time and Progeny Emergence of *Norda blitealis*

To determine the moths' emergence, the introduced larvae were allowed to feed and developed until new adult moths progeny emerged. These were counted and discard daily until no further progeny emergence is 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 the *Norda blitealis* was estimated as time from the middle of the oviposition period (estimated as a week prior to infestation) to the emergence of 50% of the F1 generation (Audi and Mukhtar, 2019)

2.7. Analyzing Susceptibility Indices

Based on the cumulative larval emergence count of F1 *Norda blitealis* to their mean developmental time on various treatments as well as the control using the methods of Dobie (1974) and Nwosu *et al.* (2015), the susceptibility of the various treated *Moringa oleifera* plants to the caterpillar attack was assessed.

The susceptibility index (SI) ranged from 0-11 (Nwosu *et al.*, 2015) where;

- 0-3 = resistant (R)
- 4-7 = moderately resistant (MR)
- 8-10 = susceptible (S)
- >11 = highly susceptible (HS)

2.8. Assessment of Damage: Larvae attack the parenchyma of tender leaves by biting and absorbing the sap, leaving only the Visual Leaves damage: transparent epidermis, bare leaf veins, drying, discoloration and necrosis caused by larvae during the period of exposure were determined based on scale rating by (Jackai and Singh, 1988) using the following formula as:

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

2.9. Statistical Analysis and Experimental design

Split plot design was adopted with various levels of Salicylic acid (SA) treatments as main plots and the spray regimes as subplots. All transformed data were subjected to Analysis of Variance ANOVA using SPSS software 20 (Version 08.12.14). Post hoc test was carried out using the Tukey test at 0.05 level of significance.

3.0. RESULTS AND DISCUSSION

3.1. Results

All salicylic acid treatments significantly ($P<0.001$) suppressed infestation of the leaf caterpillar at 2 weeks after spray than the control (Dist. H₂O). At two weeks after spraying, the moths at higher concentration (20 mg/l SA) had a lower mean percentage of progeny emergence (30.00 ± 2.16) than those at other concentrations (5 mg/l SA and 10 mg/l SA) and the control (Table 1).

Larval development was significantly ($P<0.05$) impaired in the higher treatment combination (20mg/l. SA+2-WAS). Larval development time was prolonged (20.25 ± 1.71 days) at higher treatment level than the other treatments (5mg/l and 10mg/l) and control (Table 2). Moringa plants treated with higher concentrations of SA were generally less infested and apparently more tolerant than the other treatments owing to their lower index of susceptibilities (Table 3) at higher concentration (20mg/l. SA) and the median spray regime (2-WAS) as indicated by lower progeny emergence and longer developmental period of larval *N. blitealis*

The mean percentage leaves damage also differed significantly ($P<0.001$) among the different treatment, spray regimes and the control. Damage caused by *N. blitealis* larvae was considerably high in the control (Dist. H₂O) with mean values of 83.35 ± 4.53 , 92.31 ± 1.46 and 95.31 ± 1.46 at 1-WAS, 2-WAS and 3-WAS spray regimes respectively.

Table 1: Effect of Salicylic Acid (SA) Treatments on Adults Emergence (%) of *Norda blitealis*

Hormone Treatment	No. of Moringa Stands	1-week After Spray	2- weeks After Spray	3- weeks After Spray
5ppmSA	4	73.00 ± 1.83^a	71.00 ± 0.82^b	79.00 ± 1.83^c
10ppmSA	4	57.25 ± 1.71^b	50.50 ± 2.08^c	52.50 ± 2.08^d
20ppmSA	4	33.50 ± 2.38^c	30.00 ± 2.16^d	37.50 ± 2.38^e

Dist. H ₂ O	4	80.25±1.71 ^d 0.0000	85.75±2.22 ^e 0.0000	90.75±2.22 ^f 0.0000
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Mean values with the same Superscripts are not significantly different (p<0.05)

Table 2: Effect of Salicylic Acid (SA) Treatments on larval Developmental time (days) of *Norda blitealis*

Hormone Treatment	No. of Moringa Stands	1-week After Spray	2- weeks After Spray	3- weeks After Spray
5ppmSA	4	14.50±1.29 ^a	15.50±1.29 ^b	14.50±1.29 ^a
10ppmSA	4	18.00±0.82 ^b	17.00±0.82 ^c	16.00±0.82 ^f
20ppmSA	4	19.00±1.83 ^d	20.25±1.71 ^e	19.25±1.71 ^g
Dist. H ₂ O	4	10.25±1.71 ^c	13.50±2.52 ^d	13.50±2.52 ^d
P-value		0.0000	0.0000	0.0001

Mean values with the same Superscripts are not significantly different (p<0.05)

Table 3: Effect of Salicylic Acid (SA) Exogenous Treatments on the susceptibility index of *Moringa* to infestations by *Norda blitealis*

Hormone Treatment	No. of Moringa Stands	1-week After Spray	2- weeks After Spray	3- weeks After Spray
5ppmSA	4	11.60±0.81 ^a	11.92±0.52 ^b	11.92±0.52 ^b
10ppmSA	4	10.04±0.54 ^b	10.93±0.47 ^c	11.93±0.47 ^b
20ppmSA	4	8.53±1.01 ^c	6.50±0.86 ^d	9.50±0.86 ^f
Dist. H ₂ O	4	22.68±3.25 ^d	21.11±3.03 ^e	21.11±3.03 ^e
P-value		0.0000	0.0007	0.0005

Mean values with the same Superscripts are not significantly different (p<0.05)

Table 4: Effect of Salicylic Acid (SA) Treatments on Leave Damage (%) by *Norda blitealis*

Hormone Treatment	No. of Moringa Stands	1-week After Spray	2- weeks After Spray	3- weeks After Spray
5ppmSA	4	75.99±1.92 ^a	79.15±0.92 ^b	88.15±0.92 ^c
10ppmSA	4	69.95±1.07 ^b	70.11±1.45 ^c	65.11±1.45 ^d
20ppmSA	4	46.35±1.59 ^c	37.35±1.59 ^d	41.35±1.59 ^e
Dist. H ₂ O	4	83.35±4.53 ^d	92.31±1.46 ^e	95.31±1.46 ^f
P-value		0.0001	0.0001	0.0001

Mean values with the same superscripts are not significantly different (p<0.05)

3.2. Discussions

Foliar Treatments of *Moringa oleifera* seedlings with various concentrations of salicylic acids (SA) showed varied responses to infestation by the leaf feeding caterpillar *Norda blitealis*. All salicylic acid treatments significantly (P<0.001) suppressed infestation of the leaf caterpillar at 2 weeks after spray than the control (Dist. H₂O). This can be attributed to the enhanced activation of plant defense response in the treated *Moringa* which might have triggered reaction pathways resulted in the production of variable combinations of essential metabolites. This agrees with findings of De Boer *et al.* (2004) who reported inhibition of oviposition of cabbage moths *Mamestra brassicae* by MeSA released during infestation, suggesting

that MeSA can also be detected by the attacking herbivores. A related finding reported increased metabolic production of phenolic compounds activated by Salicylic acid signals and probably enzymes systems such as aldehyde oxidases (Divol *et al.*, 2005). These might impair larval hatchability and moth emergence in all the treated plants

Exogenous foliar spray of *Moringa* with 20mg/l at 2 week after spray also led to low emergence of the moths *Norda blitealis*. The treatment also strongly impaired development of the voracious larvae which reduced their feeding activity. It is likely that the treatment appeared more compatible to the plant vascular system which translocated through the plant defense system more effectively than with

other treatment combinations. This agreed with the work of Aristo'bolo *et al.* (2007) which showed that, SA triggered synthesis of regulatory protein called Non-Expressor of Pathogenesis-Related genes (NPR1) which can be translocated through the leaves vascular system and act as antifeedant to insect herbivores.

A similar finding of Maffei *et al.* (2007) also reported that, simple phenolics (salicylates) act as antifeedant to insect herbivores and showed negative correlation between the salicylates levels and the growth of larvae of *Operophtera brumata* (L.) in Salix leaves.

Moringa plants treated with higher concentrations of SA were generally less infested and apparently more tolerant than the other treatments owing to their lower index of susceptibilities at higher concentration (20mg/l.SA) and the median spray regime (2-WAS). SA was implicated in mediating pattern triggered immunity (PTI) and effector triggered immunity (ETI) responses in monocots and dicots and is a necessary component of systemic acquired resistance (Durrant and Dong, 2004; Klessig *et al.*, 2018; Maruri-Lopez *et al.*, 2019).

The mean percentage leaves damage followed a similar trend with the higher treatment and spray regimes impacting significantly in suppression of leave damages caused by the leaf eating caterpillar, *N. blitealis*. This could be attributed to enhance tolerance capacity of the treated stand to accumulate SA at site of feeding by *N. blitealis* which might have directly act as antifeedants to the leaf eating caterpillar resulting to lower leave damage at higher treatment levels. This agrees with findings of Peng *et al.* (2004) which directly implicated SA to plant defenses of tomato in response to feeding by larvae of the cotton bollworm (*Helicoverpa armigera* Hubner). It is also in concomitant with the findings of Pasteels and Rowell-Rahier (1992) who reported that, phenolic compounds such as SA can act as deterrents and be toxic to insect herbivores.

4.0. CONCLUSION

All salicylic acid treatments significantly suppressed infestation of the leaf caterpillar than the control. Progeny emergence was considerably

low at higher concentration than with other treatments and the spray regimes. Larval development time was significantly ($P<0.05$) impaired in the higher treatment (20mg/l.SA). Moringa plants treated with higher concentrations of SA were generally less infested and apparently more tolerant than the other treatments owing to their lower index of susceptibilities at the median spray regime (2-WAS). The mean percentage leaves damage by *N. blitealis* followed similar trends among the different treatment, spray regimes and the control. This facility should be incorporated into the integrated control for effective management of Insect pest.

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