



## Histopathological effects of neem seeds extracts (*Azadirachta indica*) on adults of *Rastrococcus invadens* (Williams, 1986)

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### Abstract

*Rastrococcus invadens* is an insect infesting mango trees and causing significant damage in certain localities in Senegal. The damage affects host plants, greatly reducing mango production, thus causing enormous economic losses. Producers encounter enormous problems in the fight against this insect. The most used means of control are physical but they do not provide long-lasting results. And the other hand, chemical control can cause undesirable effects. Biological control or the use of biopesticide is therefore an alternative. In this sense, we carried out toxicity tests and showed the histological effects of neem seeds extracts (*Azadirachta indica*) on adults of *R. invadens*. The application of doses of 4%, 8%, 16%, 32% and 64% (g/ml) resulted in average mortalities of 42.92 ±10.57%; 52.59±11.41%; 54.55±10.73%; 66.22±6.91% and 100%±0.04% respectively in 24 hours compared to the control (1.033±0.05%). Histological sections showed that the product acted by destroying internal structures such as muscle tissue, cerarius and salivary glands. The results suggest that neem seed extracts has potential as a natural insecticide for the management of *R. invadens* adults and should be popularized in rural areas.

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## Introduction

In Senegal, as in most West African countries, fruits constitute food products with high nutritional and commercial value (Dembélé *et al.*, 2013). The annual production of fruits and vegetables in Senegal comes mainly from Casamance, Thiès and Dakar (Dembélé *et al.*, 2013). However, certain insects such as *Rastrococcus invadens* (Williams, 1986) pose a threat to this production and reduces its commercial value. *R. invadens* is a polyphagous species attacking many species of citrus and mango trees (Agouké *et al.*, 1988). It is a bisexual species with a pre-reproductive period in the female which can produce up to 200 larvae in its lifetime (Willink and Moore, 1988). It originated from Southeast Asia and was first identified in Senegal in 1995 (Han *et al.*, 2007). Since then, it has become widespread throughout the country and more particularly in the two most fruit-producing areas (Casamance and Thiès). Recent studies have shown morphometric (Fall *et al.*, 2018) and genetic characterization (Fall *et al.*, 2020, Seye *et al.*, 2023) of *R. invadens* in certain mango production areas in Senegal. In addition to the production of honeydew and sooty mold on the surface of leaves and fruits, this biting insect causes sap loss on the mango tree (Hala *et al.*, 2004).

This damage consequently leads to a considerable loss of mass production (Fall *et al.*, 2017) and economic loss (Fall *et al.*, 2019) in rural areas. As part of the control of these pests, physical control remains the most used by producers, compared to chemical method using synthetic products with adverse effects on humans or nontarget organisms. However, other methods such as biological control using parasitoids (Agricola *et al.*, 1989) or biopesticides based on plant extracts (Kassi *et al.*, 2021) have shown a promising method of controlling *R. invadens*. In the same way, our previous work showed that among neem derivatives (*Azadirachta indica*), seed powder in aqueous solution were effective and could be used in *R. invadens* control (Seye *et al.*, 2022). To better elucidate the toxicity of this product on *R. invadens*, it is necessary to evaluate the histological damage of neem seeds on adults.

## Materials and methods

### Animal material

*Rastrococcus invadens* is a sucking insect and attaches itself to one side of the mango leaf. The males are winged while the females are most often fixed. The individuals sampled are therefore females. The insects attached to the mango leaves were collected in the garden of the UCAD baccalaureate office: latitude 14° 41'16 N, longitude 17°28'0 and sent directly to the laboratory for testing.

### Plant material: neem seed extracts

The extracts of neem seed (*Azadirachta indica*) was obtained according to Seye *et al.*, 2002. The powder was chosen because it showed more effectiveness on the different parts of the neem. The seed were collected in the Kaolack region (Senegal). The dried powder was sieved with a diameter of 0.97 mm to obtain fine particles. The powder was conditioned in aqueous solution for 24 hours to allow good diffusion of the active ingredient in the water to be sprayed.

### Toxicity test

The sprays were carried out directly on the insects found attached to the leaves of freshly harvested mango trees of the "Sewe" variety. Based on the results of Seye *et al.*, 2022, we chose the following doses g/ml: 4%, 8%, 16%, 32% and 64% and control group was treated only with distilled water. Mortality findings were made 24 hours after spraying. The mortalities were corrected using Abbott's formula (Abott, 1925). The data collected was processed with an Excel 2013 spreadsheet. Treatments were replicated four times at different time intervals for each test and the means were reported.

### Histological study

To see the histological damage, we looked at the adults treated with the highest dose which caused 100% mortality and those of the controls in 24 hours. The insects were taken randomly from the treated batch and fixed in a carnoy solution (60% ethanol + 30% chloroform + 10% acetic acid). The technique used to carry out the sections on adults of *Rastrococcus invadens* is that described by Seye *et*

al., 2021. The staining was carried out with Hematoxylin Eosin (HE). We used an Optika brand optical microscope with an integrated camera. Images were captured with Leica DM500 digital camera and saved in JPEG format.

## Results

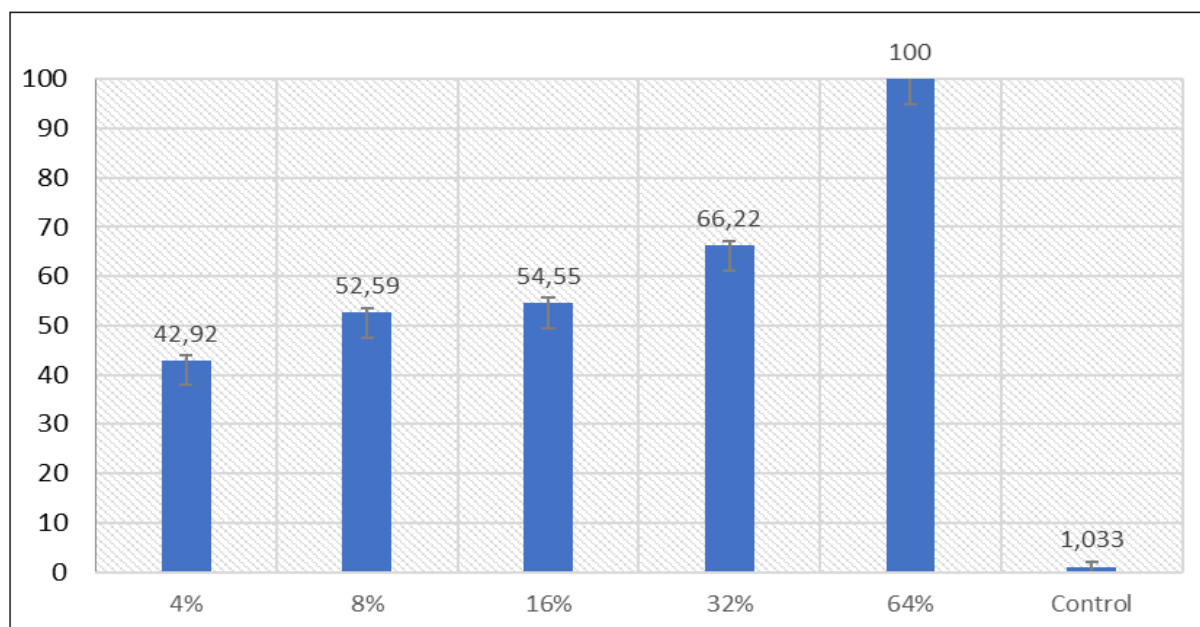
### Toxicity

The application of seed extract on *Rastrococcus invadens* adults showed an increasing mortality with increasing doses (figure 1). The doses used 4%, 8%, 16%, 32% and 64% (g/ml) against adults of *R. invadens* showed average mortalities  $42.92 \pm 10.57\%$ ;

$52.59 \pm 11.41\%$ ;  $54.55 \pm 10.73\%$ ;  $66.22 \pm 6.91\%$  and  $100 \pm 0.04\%$  respectively compared to the control  $1.033 \pm 0.05\%$  in 24 hours.

### Histological effects

In the general histological structure (Fig. 2), we note in longitudinal section a disintegration of the internal tissues of *Rastrococcus invadens* in 24 hours (Fig. 2b) compared to the control (Fig. 2a). The tissues most affected are, among others, the exocrine glands and muscle tissue. Other structures resembling maturing larvae are visible in the control (Fig. 2a) and appear to be affected by the neem product (Fig. 2b).



**Fig. 1.** Mortality of *Rastrococcus invadens* adults treated with an aqueous solution of neem powder 4% to 64% (g/ml) in 24 hours. Values are mean corrected percent mortality of four replicates with standard error (SE).

Exocrine glands and muscle was observed at high magnification (Figs 3, 4, and 5). Compared to the untreated adult (Fig. 3a) which shows the intact cerarius glands near the cuticle, we note a destruction of tissue glands for the treated adults (Fig 3b). The cuticle remains intact in places as do the conical setae.

Regarding the muscle (Fig. 4), we can observe a disorganization and destructuring muscle tissue related to the cuticle (Fig. 4a) in the treated adults compared to the control showing normal transverse and longitudinal muscle (Fig 4a).

At high magnification, we showed that the salivary glands was disintegrated (Fig. 5b) compared to the control (Fig. 5a) over time with a disorganization of the general structure. The cells become disorganized from the periphery area to the center in 24h.

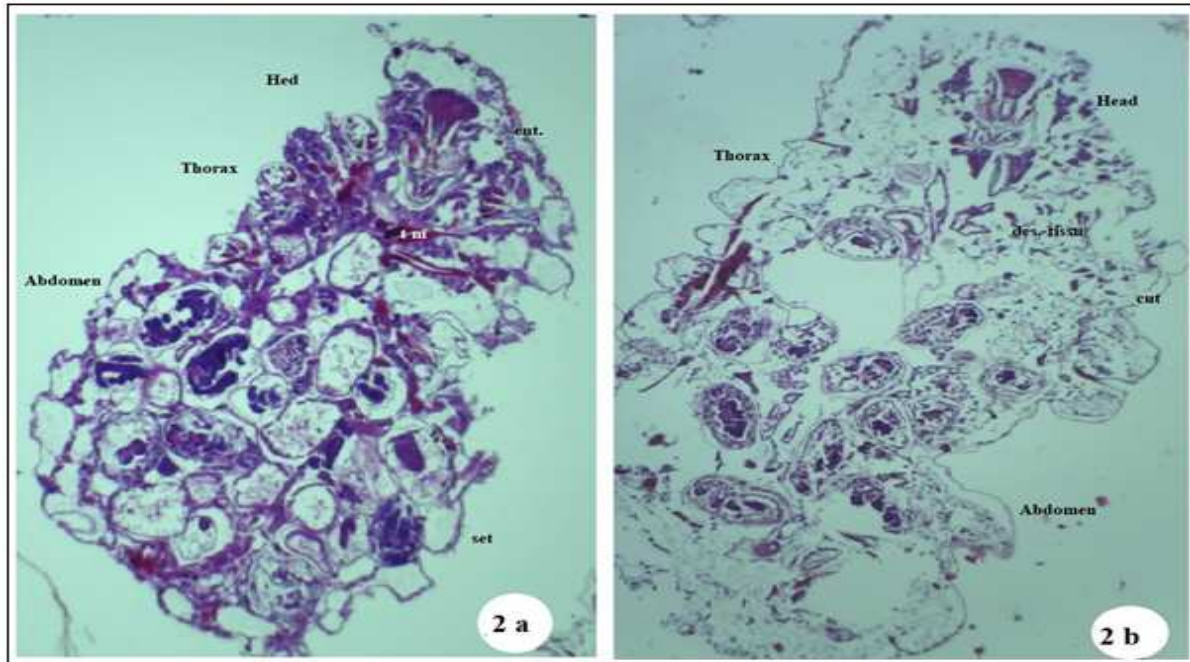
## Discussion

The application of neem seed powder (*Azadirachta indica*) to adults of *Rastrococcus invades* showed increasing toxicity following the doses applied.

The mortalities were from  $42.92 \pm 10.57\%$  to  $100 \pm 0.04\%$  compared to the control ( $1.033 \pm 0.05\%$ )

in 24h. However, we only observed 100% mortality with the 64% (g/ml) dose compared to Seye *et al.*, 2022 who obtained the same mortality with 36% (g/ml) on the same species (*R. invadens*). This difference would be due to different quality of neem

seeds that were collected in two climatic different regions or the duration of seed storage as stipulated by Abdala *et al.*, 2010. However, a lower dose could have the same effectiveness but for a longer duration after neem seed application.



**Fig. 2.** Longitudinal section (head, thorax and abdomen) of untreated adult *Rastrococcus invadens* (fig2a) and treated with neem seed aqueous solution (fig 2b) 24 hours after (x40). Cut= cutile, l m= longitudinal muscle, des. tissu= destroyed tissue, set= setae.

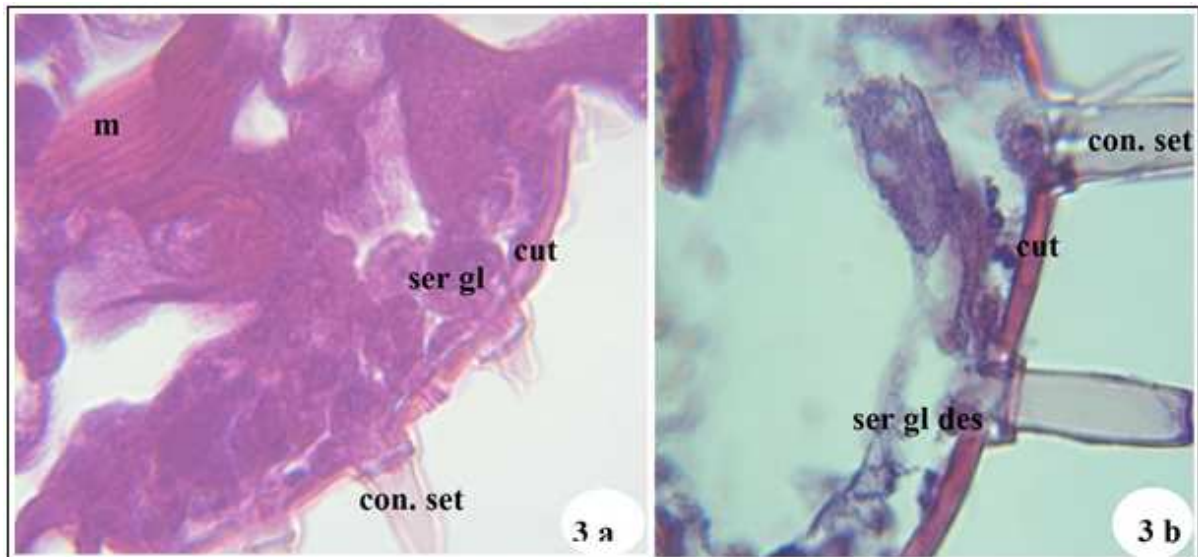
Furthermore, our results confirm the work of several authors on the toxicity of neem extracts on insects (Schmutterer, 1990; Boeke *et al.*, 2004 ; Seye *et al.*, 2006; Gnago *et al.*, 2010 ; Sané *et al.*, 2018 ; Okolo and Iledun, 2019). Neem is considered as a biocide with antifeedant effects, growth inhibition (Sami *et al.*, 2016), insect reproduction (Adhikari *et al.*, 2020; Adusei and Azupio, 2022,) or even sterility depending on the dose (Bajwa and Ahmad, 2012).

However, the work of Tulashie *et al.*, 2021, Seye *et al.*, 2022 has shown that neem extracts do not have the same effectiveness on insects depending on the part of the plant (seeds, leaf or bark). Seed are more effective than the rest of the plant.

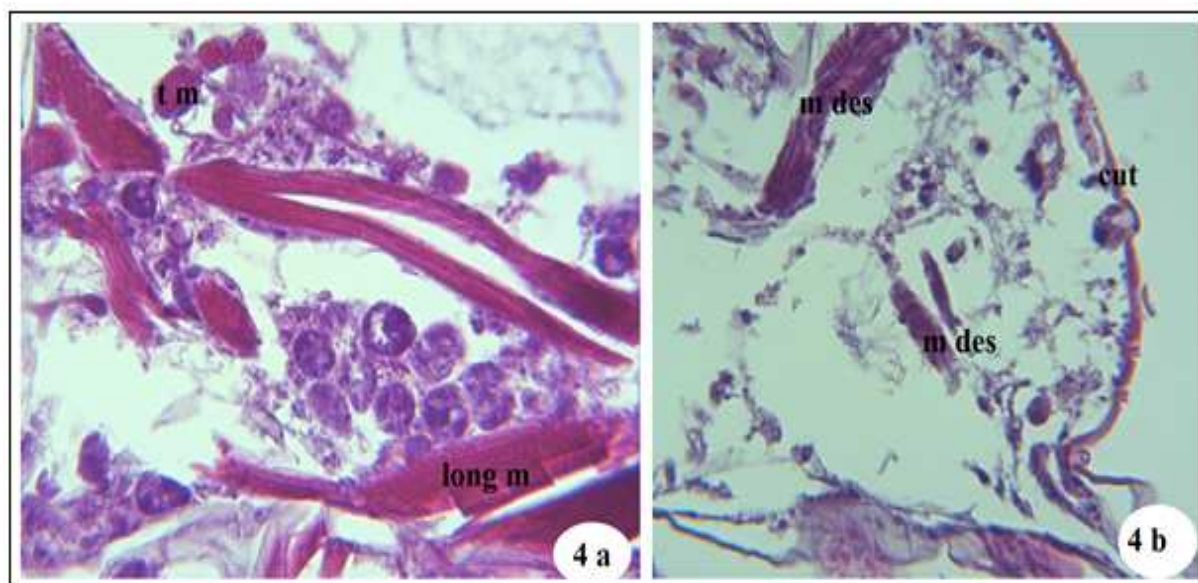
This explains why the oil extracted from neem seed shows great effectiveness in controlling harmful insects. The toxicity of the aqueous solution of neem

powder caused 100% mortality in 24 hours. This toxicity was visualized for the first time on *R. invadens* tissues in our present work. Indeed, the sections on the treated insects showed internal disorganization in 24 hours. This destruction was also observed in other exocrine glandular structures and muscle tissue. Which shows that the toxic substance, azadirachtin in particular, was able to penetrate through the cuticle to reach the internal tissues. This is more likely because the adults of *R. invadens* only feed on sap because they are sucking insects. The product therefore acted by contact and not by ingestion.

This fact directed us towards tissues other than the digestive tract unlike other insects which only feed by ingestion such as mosquito larvae (Ndione *et al.*, 2005 ; Seye *et al.*, 2006, 2021 ; Bawin *et al.*, 2016) or locusts (Nasiruddin *et al.*, 1993).



**Fig. 3.** Histological section of the peripheral zone of untreated *Rastrococcus invadens* (fig 3a) and treated with neem extract (fig 3b) 24 after (x1000). Cut= cuticle ; m=m uscle ; con. Set= conical setae ; ser. gl= cerarius gland, cer. gl. des.= cerarius gland destroyed.



**Fig. 4.** Histological section of muscle tissue from adult *Rastrococcus invadens* untreated (fig) and treated (fig) with neem powder 24 hours later (X400). Long. m= longitudinal muscle ; t m= transverse muscle, m. des. = muscle destroyed, cut= cuticle.

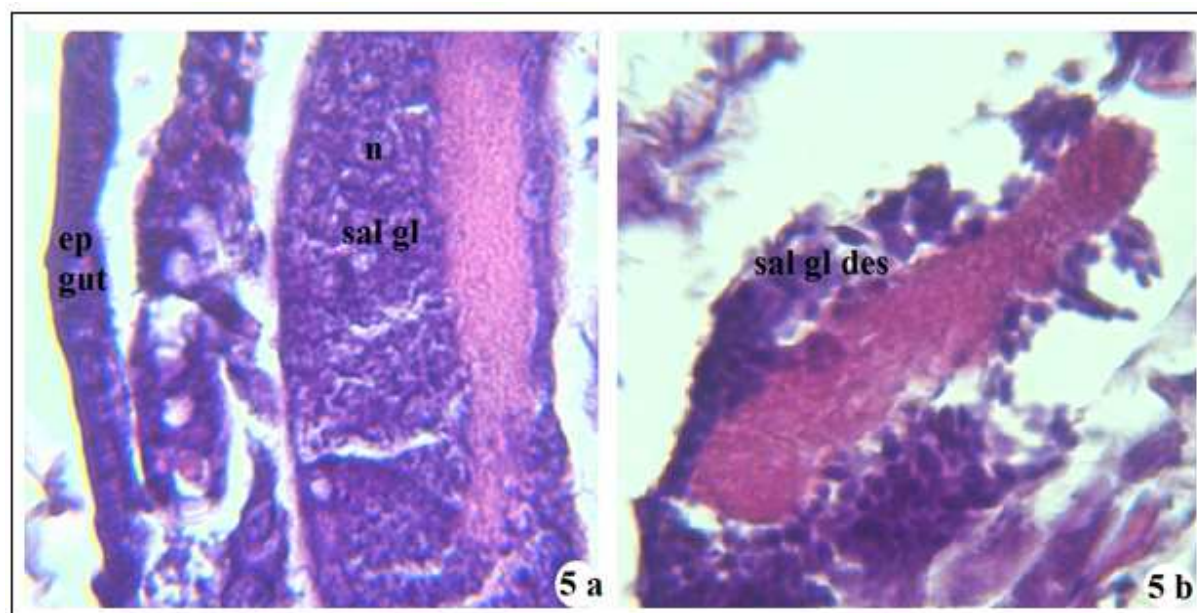
Our work has shown, among other things, a destruction of subcuticular muscles in particular. Whereas for non-sucking insects such as locusts, neem extracts act on the digestive tract through cellular disorganization (Nasiruddin *et al.*, 1993) before destroying other internal tissues.

Ont the other and, internal structures resembling maturing larvae in the abdominal cavity of adult

females are virtually destroyed 24 after treatment with neem powder. Which would confirm the work of Adhikari *et al.*, 2020 on the inhibitory effect of neem products on insect reproduction. However, we were unable to identify and examine other tissues such as those of the digestive system, nervous system and reproductive organs. This would give importance to a more histological study of the effects of neem on other tissues or internal organs. What could lead to

better show the importance of the use of neem extracts in the control of *R. invadens*. However, the both results in our present works, toxicity and

histological effects, suggest that neem extracts could be used for integrated management program for mango mealybug control in rural area.



**Fig. 5.** Histological section of adult salivary gland *Rastrococcus invadens* untreated (fig. 5a) and treated (fig. 5b) with neem powder 24 hours after (x1000). n= nucleus, sal. gl.= salivary glande ; sal. gl.des.= salivary gland destroyed ; ep. gut.= epithelial gut.

## References

**Abbott WS.** 1925. A method of computing effectiveness of insecticides. *Journal of Economic Entomology* **18(2)**, 265-267.

<https://doi.org/10.1093/jee/18.2.265a>

**Abdalla AS, Abdin EMI, Mohamed EE.** 2010. Insecticidal activities of neem (*Azadirachta indica* A. Juss) seeds under laboratory and field conditions as affected by different storage durations. *Agriculture and Biology Journal of North America.*, **1(5)**, 1001-1008.

<http://dx.doi.org/10.5251/abjna.2010.1.5.1001.1008>

**Adhikari K, Sudip B, Dikshya N, Jiban S.** 2020. Use of neem (*Azadirachta indica* A. Juss) as a biopesticide in agriculture: A review. *Journal of Agriculture and Applied Biology* **1(2)**, 100-117

<http://dx.doi.org/10.11594/jaab.01.02.08>

**Adusei S, Azupio S.** 2022. Neem: A Novel Biocide for Pest and Disease Control of Plants. *Journal of Chemistry* (2022), Article ID 6778554, p 12

<https://doi.org/10.1155/2022/6778554>

**Agouké D, Agricola U, Bokonon-GHA.** 1988 - "*Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae), a serious exotic pest of fruit trees and other plants in West Africa." *Bulletin of Entomological Research* **78**, 695-702.

**Agricola U, Agouké D, Fischer HU, Moore D,** 1989. The control of *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae) in Togo by the introduction of *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae), *Bulletin de Recherche Entomologique* **79(4)**, 671-678.

**Bajwa AA, Ahmad A.** 2012. Potential Applications of Neem Based Products as Biopesticides. *La Santé*, **3**, 116-120.

**Bawin T, Seye F, Boukraa S, Zimmer J-Y, Raharimalala FN, Ndiaye M, Compere P, Delvigne F, Francis F.** 2016. Histopathological effects of *Aspergillus clavatus* (Ascomycota: Trichocomaceae) on larvae of the southern house mosquito, *Culex quinquefasciatus* (Diptera: Culicidae), *Fungal biology* **120**, 489-499.

- Boeke SJ, Boersma MG, Alink GM, VanLoon JJA, Huis A, Dicke M, Rietjens IMCM.** 2004 - Safety evaluation of neem (*Azadirachta indica*) derived pesticides, *Journal of Ethnopharmacology*, **94**, 25-41.
- Dembélé M, Tohozin CB, Toko MI,** 2013. Inventaire des vergers de mangues dans le cercle de Bougouni au Mali. *Journal of Applied Biosciences*, **66**, 5095-5105
- Fall A, Toure M, Seye F, Ndione RD, Badiane TS, Ndiaye M.** 2017. Evaluation damage caused by *Rastrococcus invadens* (Willams. 1986) (Homoptera. Pseudococcidae) on mango in Casamance (Senegal) *Journal of Biotechnology Research* **3(12)**, 114-119.
- Fall A, Seye F, Diome T, Toure M, Ndione RD, Ndiaye M.** 2020. Genetic structuration of *Rastrococcus invadens* populations in Senegal (Casamance and Thies) *Journal of Entomology and Zoology Studies* **8(2)**, 795-801.
- Fall A, Seye F, Sarr AGRJ, Ndione RD, Toure M, Badiane TS, Mady N.** 2018. Morphometric Characterization of the Mango Tree's Mealy Cochineal, *Rastrococcus invadens*, on the Mango Tree in Senegal. *International Journal of Science and Research* **7(9)**, 1254-1260.
- Fall A, Touré M, Seye F, Ndione RD., Badiane TS, Wade B, Ndiaye M.** 2019. Evaluation des pertes causées par *Rastrococcus invadens* (Willams, 1986) (Homoptera, Pseudococcidae) sur la mangue au Sénégal (Casamance). *Journal of Applied Biosciences* **136**, 13854-13860.
- Gnago JA, Danho M, Agneroh TA, Fofana IK, Kohou AG.** 2010. Efficacité des extraits de neem (*Azadirachta indica*) et de papayer (*Carica papaya*) dans la lutte contre les insectes ravageurs du gombo (*Abelmoschus esculentus*) et du chou (*Brassica oleracea*) en Côte d'Ivoire. *International Journal of Biological and Chemical Sciences* **4(4)**, 953-966.  
<http://dx.doi.org/10.4314/ijbcs.v4i4.63035>
- Hala N, Kehe M, Allou K.** 2004. Incidence of the mango mealybug *Rastrococcus invadens* williams, 1986 (homoptera; pseudococcidae) in côte d'ivoire, *agronomie africaine* **16(3)**, 29 -36.
- Han SH, Ndiaye AB, Hemptinne JL.** 2007. Plantes-hôtes et prédateurs de la Cochenille farineuse du Manguier *Rastrococcus invadens* Williams, 1986, nouvellement introduite à Dakar, Sénégal (Hemiptera, Pseudococcidae). *Bulletin de la Société entomologique de France* **112(1)**, 121 - 125
- Kassi J-M, Koffi F, Johnson F, Moussa SA, N'Goran N'DS, Soro G, Camara B, Koné D.** 2021. *Rastrococcus invadens* Control in Mango Orchards by Using NECO, ASTOUN and FERCA Plant Extracts-Based Biopesticides. *Asian Journal of Crop Sciences* **13(1)**, 9-16.
- Nasiruddin M, Mordue AJ. (Luntz).** 1993. The effect of azadirachtin on the midgut histology of the locusts, *Schistocerca gregaria* and *Locusta migratoria*, *Tissue and Cell*, **25**, 875-884.  
[https://doi.org/10.1016/0040-8166\(93\)90036-K](https://doi.org/10.1016/0040-8166(93)90036-K).
- Ndione RD, Seye F, Ndiaye M.** 2005. Effets histopathologiques des produits de neem (*Azadirachta indica*, A. JUSS) sur les larves de *Culex quinquefasciatus* (Say, 1823) (Diptera : Culicidae), *Dakar Médical* **51(2)**, 107-112.
- Okolo ET, Iledun OC.** 2019. Insecticidal Effect of Neem (*Azadirachta Indica*) Extracts Obtained From Leaves and Seeds on Pests of Cowpea (*Vigna Unguiculata*), *Sumerianz Journal of Agriculture and Veterinary* **2(4)**, 20-28.
- Sami A J, Sehrish B, Madeeha K, Farah RS, Fayyaz R, Shakoori AR,** 2016. Effect of Crude Neem (*Azadirachta indica*) Powder and Azadirachtin on the Growth and Acetylcholinesterase Activity of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Pakistan Journal of Zoology* **48(3)**, 881-886.

**Sane B, Badiane D, Gueye MT, Faye O.** 2018 - Évaluation de l'efficacité biologique d'extrait de neem (*Azadirachta indica* Juss.) comme alternatif aux pyréthrinoides pour le contrôle des principaux ravageurs du cotonnier (*Gossypium hirsutum* L.) au Sénégal. International Journal of Biological and Chemical Sciences **12(1)**, 157-167.

**Schmutterer H.** 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual of Review Entomology **35**, 271- 297.

**Seye F, Fall A, Samb F, Diome T, Ndiaye M.** 2023. Genetic structure of *Rastrococcus invadens* populations on citrus in Senegal. International Journal of Biosciences **23(2)**, 1-16, 2023. <http://dx.doi.org/10.12692/ijb/23.2.1-16>

**Seye F, Fall A, Toure M, Ndione RD, Ndiaye M.** 2021. Histopathological effects of *Cymbopogon citratus* (lemongrass) essential oil on late third instar larvae of *Aedes aegypti* L (Diptera: Culicidae). Biology and Medicine (Aligarh) **13(1)**, 1-5.

**Seye F, Ndione RD, Ndiaye M.** 2006. Etude comparative de deux produits de neem (Huile et poudre) sur les stades préimaginaux du moustique *Culex quinquefasciatus* (Diptera : Culicidae). Afrique SCIENCE, **2(2)**, 212-225.

**Seye F, Fall A, Dia CAK, Toure M, Ndione RD, Ndiaye M.** 2022. Effect of neem derivatives (*Azadirachta indica*) on the mango mealybug (*Rastrococcus invadens*) for biological control. GSC Advanced Research and Reviews **10(03)**, 025–031. <https://doi.org/10.30574/gscarr.2022.10.3.0215>

**Tulashie SK, Francis A, John A, Enoch A.** 2021. Potential of neem extracts as natural insecticide against fall armyworm (*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) Case Studies in Chemical and Environmental Engineering **4**, 100130. <https://doi.org/10.1016/j.cscee.2021.100130>

**Willink E, Moore D.** 1988. "Aspects of the biology of *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae), a pest of fruit crops in West Africa, and one of its primary parasitoids, *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae)." Bulletin of Entomological Research **78**, 709 - 715.