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Running Title:

SAWADOGO ET AL.: *Tuta absoluta* Susceptibility to Biopesticides

Title:

Ovicidal and Larvicidal Effects of Selected Plant-Based Biopesticides on *Tuta absoluta* (Lepidoptera: Gelechiidae)[1]

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Abstract The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is a worldwide invasive pest of tomatoes (*Solanum lycopersicum* L.) that reached West Africa in 2010. Synthetic insecticides remain the most widely used method of control, but several biological alternatives are being developed. In this work, we evaluated 9 biopesticides available on the West African market for their ability to control *T. absoluta*. Using standard leaf/egg dip bioassay methodology, we compared both the ovicidal and the larvicidal activity of these biopesticides at various concentrations of active ingredients. We found that, for each biopesticide tested, the larval lethal concentrations (LC) (8.2 - 41.14 ml/l) to be lower than those necessary to stop egg hatching (26.7 - 409.7 ml/l). Two products (Bangr-Kièta : BK Bangr-Pougo: BP, formulated in powder), both based on *Azadirachta indica* A. Jussieu fruit and leaf extracts and *Khaya senegalensis* (Desrousseaux) A. Jussieu bark extract, showed high efficacy in reducing egg hatchability at their recommended doses, with a calculated control failure likelihood (CFL) reaching 0%. These two products, together with a third one (Bangr-pougo: BP) based on *Mitracarpus scaber* Zuccarini and *K. senegalensis* extracts, also showed the strongest larvicidal effects (CFL = 0%). All other tested biological insecticides showed significant efficiency but were found to be less effective at their recommended doses. Because the leafminer has developed resistance to most of the synthetic insecticide available on the market, we recommend that West African tomato producers are encouraged to use the most efficient biological products available.

Key words: Bioinsecticide, *Azadirachta indica*, *Khaya senegalensis,* control failure likelihood

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a worldwide invasive insect pest that has colonized Europe, Africa, Asia, and the Caribbean during the last two decades (Biondi et al. 2018, Son et al. 2017, Verheggen and Fontus 2019). It feeds mainly on the Solanaceae, with a preference for tomatoes (*Solanum lycopersicum* L.) (Sawadogo et al. 2022), in which the larvae cause production losses of up to 100% when no control measures are applied (Sawadogo et al. 2020a). Various methods of control have been evaluated in the past, including physical (stump removal, pruning, trapping, physical barriers), biological (predators, parasitoids), semiochemical (mass trapping with light and pheromones, mating disruption), genetic (resistant or tolerant varieties), and agricultural strategies (Chidege et al. 2018, Ferracini et al. 2019, Han et al. 2016, Jallow et al. 2020, Larbat et al. 2016, Ouardi et al. 2012, Sawadogo et al. 2021, Sohrabi et al. 2016, Zarei et al. 2019). However, chemical control with synthetic pesticides remains the most widely used, especially in the newly-invaded areas (Han et al. 2019, Sawadogo et al. 2020b). However, because of repeated applications and misuses, some leafminer populations have developed resistance to several active ingredients, making their control even more difficult (Guedes et al. 2019, Sawadogo et al. 2020b).

Biological pesticides formulated from microbial agents, chemicals of biological origin, and RNA interference (RNAi) technology have already proven to be effective against *T. absoluta*, while being less harmful to beneficials (Mansour and Biondi 2021). Different biological products based on plant extracts also have been evaluated to control the tomato leafminer, including essential oils of Zingiberaceae, Asteraceae, Cupressaceae, and Asteraceae (Alam et al. 2017, Campolo et al. 2017, Chegini et al. 2018, Umpiérrez et al. 2017), methanolic extracts from Euphhorbiaceae, Nitrariaceae, and Urticaceae (Ait Taadaouit et al. 2012), and emulsifiable formulations of Meliaceae and Rutaceae (Abd El-Ghany et al. 2018, Campolo et al. 2017). Several products are available on the West African market, even though their efficiency has barely been evaluated following proper methodology. In this work, we evaluated and compared 9 plant-based insecticides available in the West African market for their ability to control *T. absoluta* eggs and larvae.

Materials and Methods

Insects. Approximately 1200 larvae were collected between February and April 2020 in open tomato fields located in the proximity of Bobo Dioulasso (Burkina Faso) and transported to the laboratory. Insects were placed in rearing cages (80×40×40 cm) and fed tomato plants (cv. Rossol) grown without pesticides. After adult emergence, new plants were introduced in the cages and used for oviposition. After hatching, larvae were fed until their second stage (L2) when they were used for larval sensitivity tests. All rearing and bioassays were conducted at temperatures of 28 ± 3° C, relative humidity (RH) of 55 ± 15%, and under a 12:12 h photoperiod.

Bioinsecticides. All plant-based insecticides available on local markets in Burkina Faso were purchased and included in the assays (Table 1). These biopesticides were purchased from companies or producer associations based in Ouagadougou. None provided information on their labels as to ovicidal or larvicidal activity against *T. absoluta*.

Ovicidal activity. We first evaluated the ability of the different bioinsecticides to prevent *T. absoluta* eggs from hatching. Eggs were collected from the rearing cages less than 12 h after adult oviposition, keeping them attached to the tomato leaves. As per Ekesi et al. (2002), the tomato leaves with leafminer eggs were soaked for 3 s in the test solution containing 0.02% (v/v) Triton X100. A series of concentrations of each biopesticide was tested (Table 2), with 35 eggs tested for each concentration x biopesticide combination. After soaking, the tomato leaves with eggs were air-dried for 30 min under laboratory conditions and then placed in Petri dishes containing slightly moistened filter paper which was then covered and sealed with parafilm. Distilled water containing 0.02% Triton X100 was used as the control. Observations with a binocular magnifying glass were conducted each morning and evening to monitor egg hatching.

Larvicidal activity. The Insecticide Resistance Action Committee (IRAC) 022 methodology (www.irac-online.org) was followed to evaluate the larvicidal activity of the 9 biopesticides. A series of concentrations of each biopesticide was tested using 32 L2 larvae for each concentration (Table 3). Tomato leaves were soaked for 3 s in a given concentration of each of the 9 bioinsecticides with 0.02% Triton X100, after which they were air-dried for 30 min in ambient laboratory conditions. A single larva was then placed on a treated tomato leaf in a Petri dish containing slightly moistened filter paper. The control was treated with distilled water + 0.02% Triton X100. Larval mortality was assessed 72 h later. Any larva failing to display coordinated movement after 3 consecutive stimulations with a pair of forceps was considered dead.

Statistical analysis. Mortality rates were corrected using Abbott's formula for natural mortality (Abbott 1925). A probit dose-mortality response analysis was performed on the corrected data to determine the lethal concentrations for each biopesticide tested. The 95%

confidence limit (CL) was used to determine statistical significance between lethal concentrations of the biopesticides. Overlap of 95% CLs of the lethal concentrations of products indicated the lack of significant difference between the products, while non-overlap of the 95% CLs indicated statistical difference. In addition, based on the manufacturers' recommended doses, we used the formula of Guedes (2017) to calculate control failure likelihood (CFL) which is the probability that a given product used at the manufacturer recommended dose fails in controlling the pest population: CFL = 100 – (achieved mortality [%] × 100) / expected mortality (typically >80%). For all biopesticides (Tables 2, 3), the values (responses) predicted by the log (dose) / probit (mortality) model did not differ significantly from the values observed in the bioassays; thus, the probit model was found suitable for concentration/response analyses.

Results

Ovicidal activity. Bangr-kièta (BK) and Bangr-pougo (BP) powder significantly reduced egg hatch, with calculated CFLs calculated of 0% for both products (Table 2). HN and BP not formulated as a powder had low ovicidal activity with CFL levels of 71% and 64%, respectively. The remaining 5 bioinsecticides had no ovicidal impact, with CFLs >85%.

Larvicidal activity. BP not formulated as a powder, BK, and BP powder, at the manufacturers' recommended doses, eliminated >80% of *T. absoluta* larvae and exhibited the highest larval toxicity of the 9 biopesticides tested in our bioassays (Table 3). The CFLs of each of these products was 0%. Larval mortality resulting from treatment with HN, HNN^{++}, or Biopoder did not differ significantly, as indicated by overlapping 95% CLs of their respective LC_{50} values. The CFLs of these 3 products were 30%, 40%, and 47.5%, respectively. Also based on 95% CLs of their respective LC_{50} values, larval mortality following exposure to Limosain, Piol, and HNN^{+} did not differ significantly and were the least effective of the 9 biopesticides tested (e.g., CFLs range, 55 to 76.2%).

Comparison of ovicidal and larvicidal activities. Of the 9 products assayed, BK and BP powder performed best as both ovicidal and larvicidal agents against *T. absoluta*, based on their CFL values of 0% (Tables 2, 3). BP not formulated as a powder also was an effective larvicide with a CFL of 0% (Table 3). We also found that the LC_{50} and LC_{80} values calculated for each of the biopesticides were higher against *T. absoluta* eggs than L2 larvae (Tables 2, 3); thus, indicating that a higher concentration of these products is required to kill eggs than larvae.

Discussion

The 9 biopesticides we tested were formulated from several botanical sources, each of which had been previously reported to exhibit insecticidal activity (Chaieb et al. 2018, Chermenskaya et al. 2010, Doumbia et al. 2014, Fragoso et al. 2021, Kim et al. 2003, Mercier et al. 2009, Mobki et al. 2014, Murovhi et al. 2020, Ramdani et al. 2020, Sinzogan et al. 2006, Tavares et al. 2021). Furthermore, combinations of plant extracts are expected to demonstrate synergistic effects allowing for improved efficacy and control of a wide range of insects.

From our laboratory bioassays of the 9 products, we learned that Bangr-kièta (BK) and Bangr-pougo (BP) powder were the most effective ovicides against *T. absoluta* eggs, while BK, BP, and BP powder were the most effective larvicides against *T. absoluta* L2 larvae. BK and BP powder are derived from extracts of the fruits and leaves of *Azadirachta indica* A. Jussieu combined with extracts from the bark of *Khaya senegalensis* (Desrousseaux) A. Jussieu. BP is derived from extracts of *Mitracarpus scaber* Zuccarini and *K. senegalensis*.

Azadirachtin, derived from *A. indica*, is a triterpenoid that inhibits feeding, oviposition (Arnó and Gabarra 2011), and growth regulation (Schlüter et al. 1985). It is one of the main biological products currently used in leafminer control (Biondi et al. 2018, Guedes et al. 2019). It reportedly has little effect on leafminer beneficials, including adults of *Macrolophus pygmaeus* Rambur, *Trichogramma cacoeciae* Marchal , and the nematode *Steinernema feltiae* Filipjev (Amizadeh et al. 2019, Arnó and Gabarra 2011, Cherif et al. 2018). It also exhibits a similar level of efficacy as indoxacarb, metaflumizone, and abamectin (Nannini et al. 2011). However, some populations (e.g., Urla) of *T. absoluta* are not very sensitive to this product, resulting in the need for additional management tactics to control the leafminer pest (Yalçin et al. 2015).

Extracts from the bark of *K. senegalensis* are popular in traditional medicine in Africa (Drijfhout and Morgan 2010). Several triterpenoids also have been identified from the bark extracts and have proven to be potent antifeedants against *Spodoptera littoralis* (Boisduval) in bioassays. Extracts of *M. scaber* are also used in traditional medicine in West Africa and have been demonstrated to have insecticidal properties against several insect pests, including mosquito larvae (Abdullahi et al. 2011) and beetle adults in stored products (Doumbia et al. 2014).

The LC_{50}s of each of the 9 bioinsecticides tested were higher against the *T. absoluta* eggs than the LC_{50}s of the same products against the L2 larvae (Tables 2, 3). The egg shell apparently provides some protection for the developing embryo from topically-applied toxins. Furthermore, a biocidal product with antifeedant activity would target actively feeding stages of the pest insect. Thus, our results corroborate the conclusions of Campolo et al. (2017) and Chegini et al. (2018) that higher doses of the agent are required to prevent egg hatch than to kill larvae. While other botanical extracts (essential oils of *Elettaria cardamomum* (L.) Maton and *Zataria multiflora* Boiss.) have shown ovicidal effects (Chegini et al. 2018), these oils likely had fumigant properties.

Only two biopesticides (BK and BP powder) led to an acceptable ovicidal effect. For the other seven biopesticides, a higher dose than the ones recommended are likely be required to achieve acceptable management of *T. absoluta* eggs on tomatoes. Further complicating this approach is that *T. absoluta* females lay most of their eggs on the underside of leaves (Cherif et al. 2013) and are, therefore, difficult to reach with topically-applied products (Koppel et al. 2011). Control with these biopesticides should, therefore, be initiated as soon as the larvae hatch, as at this time the insect could receive the product either by contact, inhalation, or ingestion.

Even though all 9 biopesticides tested herein reportedly are larvicidal at the manufacturers' recommended doses, only BK, BP and BP powder demonstrated acceptable CFL levels in our bioassay. Increasing the recommended doses might allow for improved efficacy. Several other biopesticides in the form of essential oils have shown larvicidal effects on *T. absoluta*. For example, the essential oil of *Thymus capitatus* (L.) Hoffmanns. & Link and *Tetraclinis articulata* Vahl at 0.2 ml/L induced 80% mortality of all larval instars and 100% mortality of first-instar larvae after 1.5 h of exposure (Alam et al. 2017). Citrus peel essential oil in foliar application yielded similar results after 72 h with a concentration of 40 g/L (Campolo et al. 2017). Under greenhouse conditions, Prev-am® essential oil (made of orange oil, salt borax, and biodegradable surfactants; ORO AGRI International Ltd, Groningen, The Netherlands), as a foliar treatment, gave comparable results to lambda-cyhalothrin for the reduction of the *T. absoluta* population. This reduction is even higher when applied at half the recommended dose (10% or 0.024 g/L) in combination with the generalist predator *Nesidiocoris tenuis* Reuter (Soares et al. 2019). These and other aspects of managing *T. absoluta* on tomatoes using botanical extracts require further study with additional life stages and in field environments as well as the laboratory or greenhouse. However, our results serve as an important foundation for these additional studies.

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Table 1. Biopesticides tested in laboratory bioassays.

Manufacturer	**Trade name**	**Active ingredient**	**Recommended dose (ml/L)**
Bioprotect*	Neem oil (HN)	*A. indica* extracts	11.5
	Piol	*Capsicum annuum* L., *Allium cepa* L., *A. sativum* L., *A. indica* extracts	13.61
	Limosain	*Pinus* sp. extracts + Natural flavors, Mn, B, MaO, D-limonene	6.8
	Biopoder	*A. indica*, *Brassica* sp., *C. annuum*, *A. sativum*, *Mentha* sp. extracts	12.6
	Neem oil (HNN^{+})	*A. indica* extracts	7. 56
	Neem oil (HNN^{++})	*A. indica* extracts	7.56
Action Research Group Zems-Taaba of ADESVK**	Bangr-pougo (BP)	*M. scaber* and *K. senegalensis* extracts	200
	Bangr-kièta (BK)	Extracts of fruit and leaf of *A. indica* and extracts of bark of *K. senegalensis*	200
	Bangr-pougo (BP) formulated in powder	Extracts of fruit and leaf of *A. indica* and extracts bark of *K. senegalensis* (powder)	10 (g/l)

* Bioprotect : formulator and distributor of biopesticide certified BIO SPG and ECOCERT based in Ouagadougou, Burkina Faso

** Action Research Group Zems-Taaba of ADESVK (Association pour le Développement Economique et Sociale du Village de Koala) : Group of agricultural producers and traditional practitioners based in Sanba (Ouagadougou)

Table 2. Ovicidal effects of nine biopesticides on *T. absoluta*.

Bioinsecticides	**N**	**Number and range of concn (ml/L) tested**	**LC_{50} (ml/L)**	**95% CL**	**LC_{80} (ml/L)**	**95% CL**	**Slope±SE**	**$\chi 2$**	**Control Failure Likelihood (%)**
HN	105	3 (5-53)	26.7	20.5-46.8	43.3	31.8-88.2	0.05±0.02	0.34	71.2
Piol	140	4 (25-104)	67.3	59.1-83.4	91.6	77.8-127.7	0.04±0.01	0.46	95.6
Biopoder	175	5 (10-173)	106.2	88.9-173	163.7	129.9-482.8	0.02±0.01	0.11	89.4
Limosain	210	6 (100-680)	409.7	344.5-518.2	587.6	488.6-794.4	0.01±0.00	0.23	96.9
HNN^{+}	140	4 (10-91)	61.6	52.5-76.9	91.6	76.5-123.3	0.03±0.01	0.38	92.5
HNN^{++}	175	5 (5-85)	49.8	40-60.3	79.1	66.7-110.9	0.03±0.01	0.45	86.2
BP*	210	6 (70-480)	303.6	253.5-418.6	449.2	359.9-690.3	0.01±0.00	0.03	63.7
BK	175	5 (11-100)	61.3	47.7-71	92.9	81.6-116.7	0.03±0.01	0.1	0
BP powder	210	6 (10-150)	93.7	79.6-107.3	141.8	125.6-168.5	0.02±0.00	0.24	0

*LC_{50} and LC_{80} expressed as mg/L.

Table 3. Larvicidal effects of biopesticides on L2 *T. absoluta* larvae.

Bioinsecticides	**N**	**Number and range of concn. (ml/L) tested**	**LC_{50} (ml/L)**	**95% CL**	**LC_{80} (ml/L)**	**95% CL**	**Slope±SE**	**χ2**	**Control Failure Likelihood (%)**
HN	320	10 (3.3-40)	9.14	0.84-14.101	24.23	18.69-37.07	0.06±0.008	18.37	30
Piol	288	9 (6.7-64)	29.42	24.06-34.72	57.56	49.45-72.09	0.03±0.005	10.72	60
Biopoder	288	9 (9-80)	22.77	11.76-31.52	71.5	58.49-95.75	0.017±0.003	3.24	47.5
Limosain	384	12 (6.7-80)	25.66	13.39-35.53	69.24	54.22-107.41	0.19±0.003	15.52	55
HNN+	352	11 (3.3-50)	28.18	22.07-36.39	48.2	39.2-66.79	0.42±0.005	21.44	76.2
HNN++	352	11 (3.3-50)	8.95	4.45-12.68	31.87	26.07-42.20	0.037±0.006	13.28	40
BP	320	10 (6.7-80)	41.14	34.12-48.75	81.59	70.04-101.004	0.021±0.003	10.72	0
BK	320	10 (6.7-60)	35.99	31.97-40.30	58.10	52.32-66.32	0.038±0.004	11.86	0
BP powder*	320	10 (10.6-69)	8.20	-3.47-15.21	38.04	32.38-45.83	0.028±0.005	4.9	0

*LC_{50} and LC_{80} expressed as mg/L.