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# Toxicity, antifeedant and repellent effect of Azadirachta indica (A. Juss) and Jatropha carcus L. aqueous extracts against Plutella xylostella (Lepidoptera: Plutellidae)

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### **ABSTRACT**

Toxic effects and antifeedant rate of aqueous extract based on *Azadirachta indica* (A. Juss) and a *Jatropha carcus* L. seeds and leaves and two insecticides commonly used in vegetable fields on the larvae of the diamondback moth, *Plutella xylostella* L. were studied by bioassay using no-choice tests. The repellent effect of these products was evaluated by bioassay using choice tests. Aqueous extract of neem and jatropha seeds 80 g/L have higher toxic effect, antifeedant rate and repellent effect on *Plutella xylostella* larvae than the insecticides Décis and Cypercal in 72 hours. Antifeedant rate of aqueous neem seeds 80 g/L, aqueous jatropha seeds 80 g/L extracts and insecticides Décis 12 EC and Cypercal 50 EC were 98.99%, 97.53%, 78%, 91.30%, respectively. Antifeedant activity was increased with increasing plant extract concentrations. Aqueous neem and jatropha seeds 80 g/L were on repellent class II and insecticides Décis and Cypercal were on repellent class I. Based on the results of this study aqueous extract of neem and of jatropha seeds 80 g/L could be used to protect crops against *Plutella xylostella*.

KEYS WORDS: Azadirachta indica; Jatropha carcus; Plutella xylostella; biopesticides; larvae mortality

### 1-INTRODUCTION

The diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is oligophagous and the most destructive pest of Brassicaceae worldwide and poses particularly acute problems in tropical areas [1, 2]. The proliferation of larvae pest populations is favoured by the short duration of the life cycle, with up to 20 generations per year under tropical conditions [3] and a high reproductive potential of the females [4]. Chemical control is impaired by multiple-insecticide resistance in this species [5, 6, 7]. In some regions, *P. xylostella* larvae infect and cause 90% of crop losses despite the application of insecticides in cruciferous crops Brassica [8]. Thus, for the control of *P. xylostella*, farmers of use improperly insecticides [1]. This represents 30-50% of production costs, ahead fertilizer costs [9]. In fact, the damage caused by this pest has been estimated globally to cost US\$ 1 billion in direct losses and control costs [1, 10]. Several studies have shown that the use of insecticides is not a sustainable pest management option for farmers, as it is fraught with problems such as the improper handling of insecticides, increased cost of insecticides, reduced control efficacy and contamination of the farming environment [11]. A possible alternative to insecticides in the development of an integrated management strategy against *P. xylostella* is biological control using biopesticides based on *Azadirachta indica* and *Jatropha carcus*.

*J. curcas* extracts showed nematicidal, fungicidal effects [12]. It also exhibited insecticidal activities against moths, butterflies, aphids, bugs, beetles, flies, and cockroaches [13]. Toxicity of *J. curcas* seeds is attributed to several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters [14].

Most work showed that azadirachtin, a limonoid from the seeds and leaves of the neem tree, *Azadirachta indica* had both a potent antifeedant and insect growth regulator [15, 16].

The present study was carried out with the objective of demonstrating the biological activity of aqueous neem and jatropha seeds and leaves extracts against *P. xylostella* and to determine the efficacy and potentials of using any of these biopesticides as choice candidates in the control of this insect pest.

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#### 2- MATERIALS AND METHODS

### 2.1. Plutella xylostella

*Plutella xylostella* larvae were collected on unsprayed cabbage plots from the entomological research farm, INPHB Yamoussoukro in July 2013.

### 2.2. Insecticides, biopesticides and their preparation

### 2.2.1. Preparation of insecticides

Standard formulations of insecticides Décis (12 EC, AF- CHEM SOFACO, Côte d'Ivoire) and Cypercal (50 EC, AF- CHEM SOFACO, Côte d'Ivoire) were purchased for the study. The active ingredient of Cypercal 50 EC and Décis 12 EC are 50 g/L cypermethrin ( $C_{22}H_{19}C_{12}NO_3$ ) and 12 g/L deltamethrin ( $C_{22}H_{19}Br_2NO_3$ ), respectively. They belong to the family of synthetic pyrethroids and EC formulation. The recommended dose is 40 mL in 15 L for the Cypercal. For Décis, 50 mL of the product are added to 15 L of water. These were foliar insecticides acting by contact and ingestion. Fresh insecticide solutions were prepared when required for the bioassay (Table 1). Solutions were diluted using the formula  $C_1V_1 = C_2V_2$  [17], where  $C_1$  and  $C_2$  are concentration of first and second solution,  $V_1$  and  $V_2$  are volume of first and second solution, respectively.

### 2.2.2. Preparation of biopesticides

Biopesticides used were aqueous neem (Azadirachta indica) and jatropha (Jatropha carcus) seeds and leaves extracts at different concentrations.

For a better extraction of the active material 200, 500 or 800 grams of neem or jatropha seeds were cleaned, de-shelled and subsequently the kernels and hulls were separated manually. The kernels were grounded to fine powders. The fine powders were placed in a bucket with 10 liters of water. The content was vigorously stirred every 2 hours. After 24 hours of soaking, the solution was filtered through a piece of fabric to retain the tissue and debris on the filter and the filtrate into a clean bucket. Then, the filtrate was used for the bioassay (Table 1) [18].

For extraction of active substance of neem and jatropha leaves, the leaves were collected during day time and immediately ground in a mortar to obtain a pasty content. The pasty content was weighed and introduced into a plastic bucket containing water at a dose of 1 kg into 15 liters of water or 1 kg/10 liters. The mixture was vigorously stirred every two hours and for three days. After steeping, the solution was filtered through a clean cloth and the filtrate was used to the bioassay (Table 1) [19, 20].

### 2.3. Bioessay

Laboratory bioassays were conducted to evaluate toxicity, antifeedant and repellent effects of neem and jatropha aqueous extracts, and insecticides Décis and Cypercal at  $25 \pm 1$  °C,  $75 \pm 5$  % r. h.

### 2.3.1. Larvicidal bioassay by ingestion

In the ingestion bioassay, only a cabbage unsprayed leaves disc (5 cm diameter) were treated with insecticides Décis and Cypercal or with neem and jatropha seeds and leaves aqueous extracts at three concentrations. Both sides of cabbage unsprayed leaves disc (5 cm diameter) were dipped in 250 µl of evey concentration of Décis or Cypercal or neem or jatropha seeds and leaves aqueous extract (Table 1) diluted in 1ml of distilled water for 10 s. A cabbage leaf disc (5 cm diameter) dipped in 1.25 mL of distilled water were used for the control. After air-drying for 1 hour, leaves disc were individually placed on the filter paper in a Petri dish (9 cm diameter), into which 20 larvae were introduced. Each treatment had three replications. Mortality was assessed and the dead larvae were removed at 24 hours intervals for 3 days after initial feeding. After feeding for 48 h, the larvae were transferred into another clean Petri dish with cabbage leaf disc treated as larval food [21, 22]. The feeding area was charted on graph paper after 48 h and after 72 h after initial feeding [23].

The corrected mortality was calculated using Abbotts's [24] formula:

Mc % = 
$$\frac{\text{Mo-Me}}{100-\text{Me}}$$
 x 100, Where:

Mc: Corrected mortality (%),

Mo: number of larvae mortality in cabbage disc treated after feeding for 72 h (%);

Me: of larvae mortality in cabbage disc treated with distilled water (control) after feeding for 72 (%).

LC50 were evaluated, and the efficacy of each product in 24 h was calculated according to the formula:

E= LC50/CU

LC50: concentration (g/L) of insecticide or biopesticide required to kill half of insect's initial population at 24 hours:

CU: Concentration (g/L) of insecticide or a biopesticides used on farm.

The biopesticide or an insecticide is more effective than LC50/CU ratio is low.

The antifeedant effect of aqueous extracts of neem and jatropha seeds and leaves and insecticides Décis and Cypercal against *P. xylostella* larvae was calculated with the formula as adopted by Abivardi and Benz [25] and by Cui *et al.* [26]:

Antifeedant rate (%) =  $[(Cc-Ct)/Cc] \times 100$ ,

Table 1: Aqueous neem and jatropha extracts and of insecticides Décis and Cypercal used to bioassay

Treatment used to bioassay				
Aqueous neem extract	Aqueous jatropha extract	Insecticides		
		Décis	Cypercal	
seed extract 80 g/L (T1)	seed extract 80 g/L (T2)	0.042 g/L (T4)	0.13 g/L (T7)	
seed extract 50 g/L (T5)	seed extract 50 g/L (T6)	0.025 g/L(T'4)	0.1 g/L (T'7)	
seed extract 20 g/L (T''1)	seed extract 20 g /L (T''2)	0.016 g/L (T''4)	0.06 g/L ( T''7)	
Leaves extract 67 g/L (T3)	Leaves extract 67 g/L (T8)	-	-	
Leaves extract 35 g/L (T"3)	Leaves extract 35 g/L (T''8)	-	-	
Leaves extract 100 g/L (T'3)	Leaves extract 50 g/L (T'8)	-	-	
Jatropha seeds 80 g/L (T2) + Neem leaves 67 g/L (T3) : T		-	-	
Jatropha seeds 50 g/L (T6) + Neem leaves 67 g/L (T3): T'		-	-	

Where: Cc = consumed cabbage leaf disc in control

Ct = consumed cabbage leaf disc in Treatment.

# 2.3.2. Repellent activities of aqueous neem and jatropha seeds and leaves extracts, and of insecticides Décis and Cypercal.

The choice test assesses the repellent effect of various insecticides against insect by the method of preferential area on Whatman paper [27]. This test helps to identify a possible rejection behavior of media containing biopesticides or insecticides [28]. The Whatman paper disc (9 cm diameter) was cut into two equal parts, each with 31.80 cm<sup>2</sup> surface. Then 0.5 ml of each of the solutions thus prepared was uniformly spread on a half of the disc and the other half receives only 0.5 ml of distilled water. After one hour, the time required for complete evaporation of solvent dilution, the two halves discs were soldered using an adhesive tape. The disc of Whatman paper reconstituted was placed in a Petri dish (9 cm diameter) and 20 larvae were placed on the center of each whatman paper disc (9 cm). Three repetitions were performed for each concentration. After every two hours for eight hours, the number of insects on the treated part of Whatman paper with the product (Nt) and the number of those present on part of whatman paper disc treated only with distilled water (Nc) were counted. Repellent rate (PR) was calculated using the following formula:  $PR = (Nc-Nt) \times 100 / (Nc + Nt)$  [29] and assigned to different repellent classes ranging from 0 to V [27]: Class 0 (PR <0.1%), class I (PR = 0.1 to 20%), class II (PR = 20.1 to 40%), class III (PR = 40.1 to 60%), class IV (PR = 60.1 to 80%) and class V (PR = 80.1 to 100%).

### 2.4. Data analysis

Statistical analysis of the experimental data was performed by the Probit analysis as described by Finney [30] using XLSTAT (2013) to find out LC50. The efficacy of the different treatments was compared using the final mortalities (i.e. final cumulative mortalities). Differences in mortality rates, antifeedant rate, repellent rates were analyzed by analysis of variance (ANOVA main effect) on the threshold of 5% and average discriminated with the Student-Newman-Keuls (SNK) using the STATISTICA software version 7.1 (2005).

### 3. RESULTS

# 3.1. Toxicity of neem and jatropha seeds and leaves aqueous extracts, and of insecticides Décis and Cypercal

## 3.1.1. LC50 determination

LC50 of insecticide Décis and Cypercal were 0.11 and 1.26 g/L respectively. LC50 of aqueous extracts of jatropha seeds, neem seeds, neem leaves and of jatropha leaves were 9.32, 17.45, 116.45 and 169.95 g/L respectively. For the mixture of aqueous extracts of jatropha seeds and of neem leaves, LC50 were 12.08. The toxicity of different products were ranked more effective to least effective on *P. xylostella* larvae by comparing the LC50/CU report. Thus, all aqueous extract of neem and jatropha were more toxic by ingestion to the insecticides Décis and Cypercal. Aqueous extract of Jatropha seeds was most toxic. It was followed respectively by the mixture of aqueous jatropha seeds extract and neem leaves, aqueous neem seed extract, aqueous neem leaves extract and by aqueous jatropha leaves extracts (Table 2).

**Table 2:** LC50 and efficacy order of aqueous extracts based on neem and jatropha, and of insecticides Décis and Cypercal to *P. xylostella* larvae in the ingestion toxicity test

Efficacy order	Treatment	LC50 at 24 h (g/L)	Concentration (g/L) use on farm (CU)	LC50/CU
1	Aqueous jatropha seeds extract	9.32	50	0.18
2	Aqueous jatropha seeds extract + Aqueous neem leaves extract	12.08	50	0.24
3	Aqueous neem seeds extract	17.45	50	0.35
4	Aqueous neem leaves extract	116.65	67	1.74
5	Aqueous jatropha leaves extract	169.95	67	2.53
6	Décis 12 EC	0.11	0.042	2.62
7	Cypercal 50 EC	1.26	0.13	9.69

### 3.1.2. Toxicity of aqueous neem and jatropha extracts after 72 hours

# Toxicity of aqueous neem and jatropha seeds extract and of insecticides Décis and Cypercal to *P. xylostella* larvae

The mortality of *P. xylostella* larvae consuming cabbage discs impregnated with aqueous neem and jatropha seed extracts 20 g/L, 50 g/L or 80 g/L, or with insecticides Décis and Cypercal were lowered the first day and did not exceed 30% regardless of the product used. The aqueous extracts from neem seeds 20 g/L and jatropha 20 g/L induced the highest mortality in 24 hours. These mortality rates were 25.88% and 28.95% respectively. The mortality rates were respectively followed by those of insecticides Décis (20.61%) and Cypercal (17.36%), aqueous jatropha seeds extract 50 g/L (15.09%), aqueous jatropha seeds extract 80 g/L (10.18%) and those of aqueous neem seeds extract 80 g/L (10%) and 50 g/L (3.33%). After 48 hours feeding, *P. xylostella* larvae mortality in the aqueous jatropha seeds extract 20 g/L (63.40%) and 50 g/ L (60.06%) were the highest. These mortality rate of *P. xylostella* larvae were followed respectively by those of aqueous neem seeds extract 20 g/L (59.59%), insecticide Cypercal (58.82%), aqueous jatropha seeds extract 80 g/L (55.77%), aqueous neem seed extract 80 g/L (53.92%), Décis (47.71%) and aqueous neem seed extract 50 g/L (34.75%). After 72 hours feeding, aqueous neem seed extract 80 g/L produced high mortality (66.39%) to the larvae than the aqueous neem seeds extracts 20 g/L (62.28%) and 50 g/L (54.62%). The aqueous jatropha seeds extract 80 g/L (71.83%) induced high mortality to *P. xylostella* larvae compared to other jatropha seeds extracts 20 g/L (68.42%) and 50 g/L (56.63%). The Cypercal induced high mortality rate (69.06%) than Décis (61.26%).

Aqueous neem and jatropha seed extracts 20 g/L and 80 g/L mortality rate were greater than that of the insecticide Décis after 72 hours feeding. Only aqueous jatropha seeds extract 80 g/L induced high mortality to P. xylostella larvae than Cypercal after 72 hours feeding (Figure 1).

# Toxicity of aqueous neem and jatropha leaves extracts, and of neem leaves and jatropha seeds, and of insecticides Décis and Cypercal to *P. xylostella* larvae

The first day of *P. xylostella* feeding, the aqueous jatropha leaves extract 67 g/L (20.79%) produced higher mortality to *P. xylostella* larvae than the insecticides Décis (20.61%) and Cypercal (17.37%), aqueous extracts T' (10.26%), T (8.68%), and aqueous neem leaves extracts 67 g/L (1.67%). After 48 hours feeding, the mortality rate of each tested product increased and was higher for the aqueous jatropha leaves extract 67 g/L (61.66%). This mortality rate obtained with the aqueous jatropha leaves extract 67 g/L on the second day were respectively followed by those of Cypercal (58.82%), aqueous extract T' (53.49%), Décis (47.71%), aqueous extract T (44.23%) and by aqueous neem leaves extract 67 g/L (38.34%). On the third day of experiment, the mortality rate of *P. xylostella* larvae obtained for aqueous extracts T' was 61.58% and was similar to that of insecticide Décis (61.26%). These mortality rates of *P. xylostella* larvae remain below those of insecticide Cypercal (69.05%) and those of the aqueous jatropha leaves extract 67 g/L (69.29%). Moreover, the mortality rate of *P. xylostella* larvae were the lowest for the aqueous extract of neem leaves 67 g/L (52.26%) and aqueous extract T (47.42%) (Figure 2).

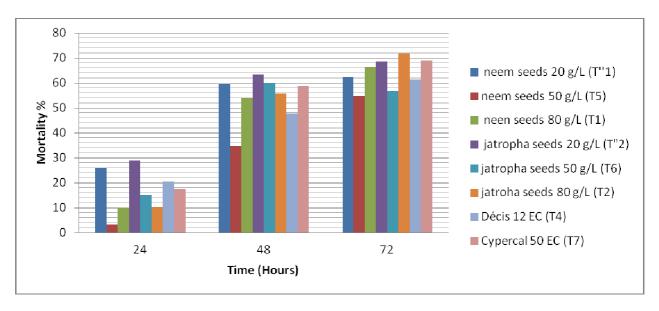
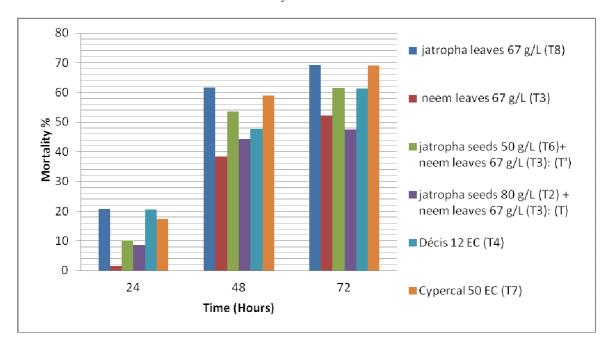


Figure 1: Ingestion toxicity of aqueous neem and jatropha seeds extract and of insecticides Décis and Cypercal on *P. xylostella* larvae.



**Figure 2**: Ingestion toxicity of aqueous neem and jatropha leaves extracts, and of neem leaves and jatropha seeds, and of insecticides Décis and Cypercal on *P. xylostella* larvae.

# 3.2. Antifeedant and repellent activities of jatropha and neem seeds and leaves aqueous extracts, and of insecticides Décis and Cypercal.

### 3.2.1. Antifeedant activity

Aqueous extracts, of neem seeds 50 g/L and 80 g/L, of jatropha seeds 80 g/L and the aqueous extract T' significantly reduced the consumption of cabbage leaves by *P. xylostella* larvae. As the aqueous neem leaves extract 67 g/L, aqueous extract T and Cypercal, moderately restricted food intake by *P. xylostella* larvae. The aqueous jatropha seeds extract 50 g/L, aqueous jatropha leaves extract 67 g/L and Décis had low antifeedant rate. The antifeedant rate of aqueous neem seeds extracts 80 g/L (98.19%) and 50 g/L (97.53%) to *P. xylostella* larvae were high. The antifeedant rate decreased slightly when the concentration of the extract was 50 g/L and it was very lowered when cabbage leaf disc were treated with aqueous neem seed extract 20 g/L (39.65%). As for the aqueous neem leaves extracts 67 g/L, its antifeedant rate was 93.61%.

Aqueous jatropha seeds extract 80 g/L had higher level of antifeedant rate (97.53%) than aqueous jatropha seeds extract 50 g/L (86%) and 20 g/L (54.43%). Aqueous jatropha leaves extracts 67 g/L (T8) induced 83% antifeedant rate and were lower than those of aqueous jatropha seeds extract 80 and 50 g/L (Table 3).

### 3.2.2. Repellent activity

Witnesses' paper and paper treated with different products were visited by *P. xylostella* larvae. The results indicate that the aqueous extracts based on neem and jatropha seeds and leaves act as potential repellent activities to *P. xylostella* larvae. In fact, the aqueous neem seeds extracts 80 g/L (T1) (36.67%) and 50 g/L (T5) (36.67%), and of jatropha seeds 80 g/L (T2) (24.17%) and 50 g/L (T6) (36.67%), and aqueous extracts of jatropha leaves 67 g/L (T8) (27.5%) and T' (30.00%) were on repellent class II. Their repellency activities were superior to those of insecticides Décis (18.33%) and Cypercal (6.67%), aqueous neem leaves extracts 67 g/L (T3) (19.17), T (8.33), T' 1 (5.83) and T' 2 (14.17) which were on repellent class I (Table 3).

#### 4. DISCUSSION

Neem and jatropha seeds and leaves aqueous extract, and insecticides Décis and Cypercal have showed remarkable effect on *P. xylostella* larvae as toxic, antifeedant, and repellent compounds. However, LC50 values to *P. xylostella* larvae determined at insecticides Décis and Cypercal ingestion were high compared to the concentrations used on field. The toxicity of insecticides Décis and Cypercal is related to their neurotoxic activity. In fact, pyretroids cause prolonged membrane depolarization leading to repetitive nerve firing by binding to sodium channels and keeps the channel open [31]. According to Al-Sayeda [32], insecticides caused cholinesterase inhibition responsible for the inactivation of acetylcholine at neuromuscular junctions. Acetylcholinesterase (AChE) is a key enzyme in the cholinergic synapses where it rapidly terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. Insecticides are substrates of AChE and their hydrolysis results in the phosphorylation of the active serine followed by dephosphorylation [33]. This dephosphorylation is very long, synaptic transmission remains blocked, whereas deacetylation of the acetylated enzyme by its natural substrate acetylcholine is a rapid process [34]. Consequently, blockage of AChE by insecticides leads to the death of the insect.

The high value of LC50 in bioessay showed that farmers use large quantities of insecticides to protect their crops from *P. xylostella* damage. Indeed, intensive use of insecticides in its control has led to this pest developing resistance to a range of insecticides. This was in accordance with Sayyed and Wright [5], who reported that populations of *P. xylostella* collected in fields treated in Malaysia had high resistance to cypermethrin and

**Table 3**: Antifeedant rate, repellent effect and repellent class of aqueous neem and jatropha extract, and of insecticides Décis and Cypercal on *P. xylostella* larvae

Treatment	Antifeedant rate	PR (%)	class
Neem seeds 80 g/L (T1)	$98.19a \pm 1.26$	36.67a	II
Jatropha seeds 80 g/L (T2)	$97.53a \pm 1.46$	24.17abc	II
Neem leaves 67 g/L (T3)	93.61ab ±1.68	19.17abc	I
Décis 12 EC (T4)	$78.00c \pm 1.15$	18.33abc	I
Neem seeds 50 g/L (T5)	$97.53a \pm 1.14$	36.67a	II
Jatropha seeds 50 g/L (T6)	$86.00bc \pm 2.74$	36.67a	II
Cypercal 50 EC (T7)	$91.30ab \pm 3.02$	6.67c	I
Jatropha leaves 67 g/L (T8)	83.00bc±2.81	27.50abc	II
Jatropha seeds 80 g/L (T2) + Neem leaves 67 g/L (T)	94.11ab ±2.63	8.33c	I
Jatropha seeds 50 g/L (T6) + Neem leaves 67 g/L (T')	$97.86a \pm 0.97$	30.00ab	II
Neem seeds 20 g/L (T"1)	$39.65e \pm 10.91$	5.83c	I
Jatropha seeds 20 g/L (T''2)	$54.43d \pm 12.03$	14.17bc	I
p	0.00	0.00	-

Means followed by the same letter in a same column are not significantly different at P < 0.05 level (Newman-Keuls test).

Deltamethrin compared to untreated population in the laboratory. Similar results were reported by Khaliq *et al.* [35], who showed that LC50 values increased to *P. xylostella* in several localities of Pakistan. According to these authors, LC50 increased from 0.19 to 1.88 mg/L for cypermethrin and 0.31 to 2.64 mg/L for deltamethrin. Attique *et al.* [36] and Zhao *et al.* [6] also showed that the resistance of field populations of *P. xylostella* gradually increases. These resistance developed by the insects could be link to degradation and modification of insecticides target such as acetylcholinesterase [33, 34].

The aqueous extracts of neem seeds and jatropha at high concentrations 80 g/L and 50 g/L had higher toxicity, antifeedant and repellent effect than Cypercal and Décis. These highs toxicity, repellent and antifeedant effects of biopesticides based on neem and jatropha seeds aqueous extract led to the high concentrations of bioactive compounds on seed which induced high biological effect on *P. xylostella* larvae. According to Trematerra and Sciarretta [37], repellent and antifeedant effects of aqueous neem and seeds extracts were responsible for insect's larvae and adults' mortality. The compounds of these biopesticides neem and jatropha would act on insect's chemoreceptor and prevent food intake. According to Mordue-Luntz *et al.* [38]

Azadirachtin stimulates specific 'deterrent' cells in chemoreceptors and also blocks the firing of 'sugar' receptor cells, which normally stimulate feeding. Jatropha aqueous extracts contain protease inhibitors [39, 40] and secondary metabolites of neem affected the production of digestive enzymes such as amylase, glucosidases, lipases and proteases [41]. These biopesticides were on repellent class II. When these biopesticides based on neem and jatropha seeds were diluted (20 g/L), their repellency effect decreases (repellent class I) and its induced high consumption of cabbage leaves discs treated with these biopesticides. Consequently, it's induced high mortality of *P. xylostella* larvae.

The aqueous neem extracts contained azadirachtin, the Nimbin, the salanine and sterol [42] and other substances in particular saanine and meliantriol which have an effect on insect biology [43]. These compounds of neem on the cabbage leaf disc reduced the consumption of P. xylostella larvae. Azadirachtin can affect the secretory function of neuroendocrine cells in insects [44]. The incorporation of azadirachtin in the food hoppers greatly reduces the enzymatic activity of α-amylase [45], which could disturb the mechanism controlling the production of trypsin by the cells of the wall midgut [46]. When azadirachtin enters in the body of the larvae, it is mediated by its binding to the ecdysone receptor (EcR), in the presence of a heterodimeric partner, ultraspiracle protein (USP) [47, 48]. The activity of ecdysone is suppressed and the larva is unable to mutate and remain in the larval stage and eventually died [48]. According to Rembold and Banerjee [49], azadirachtin causes accumulation of serotonin in neuroendocrine organs, interferes with neurotransmitter and this causes the inhibition of the release of ecdysone. These neem extracts with high concentrations reduce the release of phosphorus for energy metabolism and the rate of transport of metabolites and this could be the cause of the insecticidal activity of aqueous neem seed extract on insects [50, 51]. According to these authors, these compounds modify the activity of adenosine triphosphatases (ATPase) in insects. The incorporation of neem bioactivities substances, azadirachtin, salanine and nimbin in an artificial diet significantly reduced consumption and relative growth of fourth instar of Spodoptera litura (F.) larvae compared with controls [52]. These secondary metabolites had toxic and antifeedant effects in insects [53]. According to Rangarajan et al. [54] and Bouchelta et al. [55], the salanine is a glycoalkaloid which exerts repellent and toxic effects on insects. Thus, these products may allow plants to restrict the number of phytophagous insect species able to grow at their expense.

The toxicity of the aqueous extracts of jatropha was led to the presence of several compounds that have toxic effects of insecticides on insects. Numerous studies have shown that extracts of jatropha seeds and leaves contain curcin with lectin activity, phorbol ester [14, 56], the inhibitor of trypsin and saponins [56, 57]. These toxic compounds have a negative impact on insect biology and would be responsible for the mortality of larvae and adult insects. Curcin was more concentrated in jatropha seeds than in the leaves and this protein inactive ribosomes (RIP) [58, 59]. According to Millard and Leclaire [59] and Insanu *et al.* [60], curcin blocks translation of proteins in the liver by inactivating ribosomal sub-units. The small amount of ester phorbol ester aqueous extracts of jatropha would be in synergy with curcin and amplify toxicity curcin. According to Goel *et al.* [61], phorbol esters are inserted in the cell membrane through which the active receptor occupancy protein kinase C (PKC) found in all tissues but more concentrated in insect neuronal tissues. This enzyme plays an essential role in signal transduction that regulates cell growth and differentiation [40, 61]. Its activation leads to proliferation and cell differentiation manifested by inflammation [40, 61]. Consumption of phorbol ester would irritate the mucous membrane and have a hemolytic activity [40].

The protease inhibitors contained in the aqueous extracts jatropha reduced the digestibility of plant tissues by pests and are responsible for inhibiting the growth of insects. Rackis and Gumbmann [39] and Nesseim *et al.* [40] have showed that protease inhibitors reduce the growth and development of insects by excessive losses of undigested fecal proteins. Saponins contained in the aqueous extracts of jatropha were very bitter and had reppellent and toxic effects on insects [55]. Its Ingestion caused stunting and reduced ration energy value [40].

### **5- CONCLUSION**

Our results demonstrate aqueous neem and jatropha seeds 50 and 80 g/L had high toxic, antifeedant and reppellent effect to *P. xylostella* larvae. These biopesticides based on neem and jatropha can be used for *P. xylostella* control.

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#### REFERENCES

- 1. Talekar, N.S. and A.M. Shelton, 1993. Biology, Ecology and management of Diamondback moth. *Ann. Rev. Entomol.*, 38: 275-301.
- 2. Seenivasagan, T. and N.A.V. Paul, 2011. Electroantennogram and flight orientation reponse of *Costesia plutallae* to hexane extract of cruciferous host plants and larvae of *Plutella xylostella*. *Entomol. Res.*, 41: 7-17.
- 3. Vickers, R.A., M.J. Furlong, A. White and J.K. Pell, 2004. Initiation of fungal epizootics in Diamondback moth populations within a large field cage: proof of concept of auto-dissemination. *Entomol. Exp. Appl.*, 111: 1-17.
- 4. Justus, K.A., I.M. Dosdall and B.K. Mitchell, 2000. Oviposition by *Plutella xylostella* (Lepidoptera: Plutellidae) and effect of phylloplane waxiness. *J. Econ. Entomol.*, 93: 1152-1159.
- 5. Sayyed, A.H. and D.J. Wright, 2006. Genetics and evidence for an esterase associated mechanism of resistance to Indoxacarb in field population of diamondback moth (Lepidoptera: Plutellidae). *Pest Manag. Sci.*, 62: 1045-1051.
- 6. Zhao, J. Z., H.L. Collins, Y.X. Li, R.F. Mau, G.D. Thompson and M. Hertlein *et al.*, 2006. Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb and emamectinbenzoate. *J. Econ. Entomol.*, 99: 176 181.
- 7. Zhou, L., J. Huang and H. Xu, 2011. Monitoring resistance of field populations of diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) to five insecticides in South China: A ten-year case study. *Crop Prot.*, 30: 272-278.
- 8. Cartea, M. E., G. Padilla, M. Vilar and P. Velasc, 2009. Incidence of the major *Brassica* pests in northwestern Spain. *J. Econ. Entomol.*, 102: 767-773.
- 9. Zalucki, M.P., A. Shabbir, R. Silva, D. Adamson, S.S. Liu and M.J. Furlong, 2012. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella*: Just how long is a piece of string? *J. Econ. Entomol.*, 105: 1115-1129.
- 10. Grzywacz, D., A. Rossbach, A. Rauf, D.A. Russel, R. Srinivasan, A.M. Shelton, 2010. Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. *Crop Prot.*, 29: 68-79.
- 11. Dobson, H., J. Cooper, W. Manyangarirwa, J. Karuma and W. Chiimba, 2002. Integrated Vegetable Pest Management: safe and sustainable protection of small scale brassicas and tomatoes. Natural Resources Institute (NRI), Kent, UK: 179.
- 12. Sharma, N. and P.C. Trivedi, 2002. Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum*. *Asian J. Exp. Sci*, 16: 21-28.
- 13. Wink, M., C. Koschmieder, M. Sauerwein and F. Sporer, 1997. Phorbol esters of *Jatropha curcas*biological activities and potential applications. In: Gübitz GM, Mittelbach M, Trabi M editors. Bio-fuel and industrial products from *Jatropha curcas*. Dbv-Verlag Univ. Graz.
- 14. Makkar, H.P.S., K. Becker, F. Sporer and M. Wink, 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J. Agri. Food Chem.*, 45:3152-3157.
- 15. Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica. Annu. Rev. Entomol.*, 35: 271-291.
- 16. Koul, O., 2004. Neem: a global perspective: 1-19. *In* O. Koul and S. Wahab [eds.], Neem: today and in the new millennium. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- 17. Gupta, S., S. Jafar, V. Jaiwal, P. R. Singh and M. Maithani, 2011. Review on titrimetric analaysis. *Int. J. Compréhensive Pharm.*, 2: 1-6.
- 18. Anonyme, 2003. Les savoirs traditionnels au profit de la lutte contre les maladies et ennemis des cultures. Ecole instrument de paix, Niger : 11 p.
- 19. N'Diaye, M. and M. Seck, 1997. Effet de l'extrait aqueux des feuilles de neem (*Azadirachta indica* Juss) sur la population de thrips et le rendement du niebe (*Vigna unguiculata*), rapport de stage au Sénégal : 28 p.
- 20. Padonou, S.C., 2008. Analyse comparée du revenu et de sa distribution entre les producteurs de tomate utilisant les biopesticides et les pesticides chimiques en zone periurbain du Sud Benin. Mémoire de Diplôme d'ingénieur agronome, Université de Parakou: 8 p.

- Shelton, A.M. and J.A. Wyman, 1992. Insecticide resistance of DBM in North America. In: Talekar NS (ed.)
   DBM and Other Crucifer Pests Proceedings of second international workshop. AVRDC, Tainan, Taiwan. pp
   447-454.
- 22. Shi, Z-h., S-j. Guo, W-c. Lin and S-s. Liu, 2004. Evaluation of selective toxicity of five pesticides against *Plutella xylostella* (Lep: Plutellidae) and their side-effects against *Cotesia plutellae* (Hym: Braconidae) and *Oomyzus sokolowskii* (Hym: Eulophidae). *Pest Manag. Sci.*, 60: 1213-1219.
- 23. Ansari, M.S., T. Ahmad, H. Ali, 2010. Effect of Indian mustard on feeding, larval survival and development of *Plutella xylostella* at constant temperatures. *Entomol. Res.*, 40: 182-188.
- 24. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
- 25. Abivardi, C. and C. Benz, 1984. Tests with the extracts of 21 medicinal plants for antifeedant activity against larvae of *Pieris brassicae* L. (Lep., Pieridae). *Mitt. Schweiz. Entomol. Ges.*, 57:383-392.
- 26. Cui, J., M-L. Li and M-S. Yuan, 2013. Semisynthesis of N-acyl homoserinelactone derivatives and the antifeedant activity against *Mythimna separate*. *J. Environ. Sci. Health, Part B*, 48: 671- 676.
- 27. McDonald, L.L., R.H. Guy and R.D. Speirs, 1970. Preliminary evaluation of news candidates materials as toxicants, repellents and attractants against stored product insects. Marketing Res. Rep. n° 882. Wanshington: *Agric. Res. Service*, US/ Dept of Agric., 183 p.
- 28. Sauvion, N., 1995. Effets et modes d'action de deux lectines à mannose sur le puceron du pois, *Acyrthosiphon pisum* (Harris). Potentiel d'utilisation des lectines végétales dans une stratégie de création de plantes transgéniques résistantes aux pucerons. Thèse de Doctorat, Institut National des Sciences Appliquées de Lyon, France : 195 p.
- 29. Alzouma, I., 1990. Les problèmes de la post-récolte en Afrique sahélienne. *In*: Foua Bi K. and Philogene J. (eds.). Actes de séminaire International de la post-récolte en Afrique. Abidjan, Côte d'Ivoire, 22-27.
- 30. Finney, D.J., 1971. Statistical Methods in Biological Assay, 2<sup>nd</sup> edition. London: Griffin, 333 p.
- 31. Nehare, S., B.S. Ghodki, G.K. Lande, V. Pawade and A.S. Thakare, 2010. Inheritance of resistance and cross resistance pattern in indoxacarb-resistant diamondback moth *Plutella xylostella* L. *Entomol. Res.*, 40: 18-25.
- 32. Al-Sayeda, H., 2007. Transfert d'un insecticide systémique, l'imidaclopride, chez la tomate : implication du transport phloémien. Thèse de Doctorat, Institut National Polytechnique de Toulouse, France, 143 p.
- 33. Aldridge, W.N., 1950. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl p-nitrophenyl thiophosphate (E605) and analogues. *Biochem. J.*, 46:451-460.
- 34. Shi A.M., A. Lougarre, C. Alies, I. Frémaux, Z. H. Tang, J. Stojan and D. Fournier, 2004. Acetylcholinesterase alterations reveal the fitness cost of mutations conferring insecticide resistance. *BMC Evolutionary Biology*, 4(5):1-8.
- 35. Khaliq, A.M., N.R. Attique, A.H. Sayyed, 2007. Evidence for resistance to pyrethroids and organophosphate in *Plutella xylostella* (Lepidoptera: Plutellidae) from Pakistan. *Bull. Entomol. Res.*, 97: 191-200.
- 36. Attique, M.N., R.A. Khaliq and A.H. Sayyed, 2006. Could resistance to insecticides in *Plutella xylostella* (Lepidoptera: Plutellidae) be overcome by insecticide mixtures. *J. Appl. Entomol.*, 130: 122-127.
- 37. Trematerra, P. and A. Sciarretta, 2002. Activity of chilli, *Capsicum annuum* L. *var. acuminatum*, on stored product insects *Oryzaephilus surinamensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). *IOBC/wprs Bull.*, 25 (3): 177-182.
- 38. Mordue-Luntz A.J., A. Zounos A., I.R. Wickramananda and E.J. Allan, 1995. Neem tissue culture and the production of insect antifeedant and growth regulatory compounds. BCPC Symp. Proceed, 63: 187-194.
- 39. Rackis, J.J., M.R. Gumbmann, 1981. Protease inhibitors: physiological properties and nutritional significance. *In:* Ory R.L. *Antinutrients and natural toxicants in foods.* Westport, CT, USA: Food and Nutrition Press.
- 40. Nesseim, T.D.T., M. Fillet, G. Mergeai, A. Dieng and L. Jean-Hornick, 2012. Principes toxiques, toxicité et technologie de détoxification de la graine de *Jatropha curcas* L. (synthèse bibliographique). *Biotechnol. Agron. Soc. Environ.*, 16(4): 531-269.
- 41. Senthil-Nathan, S., 2013. Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. *Front Physiol.*, 4: 359.

- 42. Faye, M., 2010. Nouveau procédé de fractionnement de la graine de neem (*Azadirachta indica* A. JUSSI) Sénégalais : production d'un biopesticide d'huile et de tourteau. Thèse de doctorat de l'Université de Toulouse, France, 228 p.
- 43. Philogène, B.J.R., 1991. L'utilisation des produits naturels dans la lutte contre les insectes : problèmes et perspectives. *La lutte anti-acridienne*. *AUPELF-UREF* (ed.). Paris : 269-278.
- 44. Garcia, E., N. Luz, P. Azambuja and H. Rembold, 1990. Azadirachtin depresses the release of prothoracicotropic hormone in *Rhodnius prolixus* larvae: evidence from head transplantations. *J. Insect Physiol.*, 36: 679-682.
- 45. Rharrabe, K., H. Amri, N. Bouayad and F. Sayah, 2008. Effects of azadirachtin on post-embryonic development, energy reserves and α-amylase activity of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *J. Stored Prod. Res.*, 44: 290-294.
- 46. Timmins, W. and S. Reynolds, 1992. Azadirachtin inhibits secretion of trypsin in midgut of *Manduca sexta* caterpillars: reduced growth due to impaired protein digestion. *Entomol. Exp. Appl.*, 63: 47-54.
- 47. Orlowski, M., M. Szyszka and A. Kowalska, 2004. Plasticity of the Ecdysone Re-ceptor DNA Binding Domain [J]. *Molecular Endocrinology*, 18(9): 2166-2184.
- 48. Zhao, J-c., T-m. Wu, L-h. Liu, Y. Wang and L. He, 2014. EcR-RNAi and azadirachtin treatments induced the abnormal proleg development in *Spodoptera litura*. *J. East China Normal University* (Natural Science), 1: 133-141.
- 49. Banerjee, S. and H. Rembold, 1992. Azadirachtin a interferes with control of serotonin pools in the Neuroendocrine System of Locusts [J]. Naturwissenschaften, 79: 81-84.
- 50. Senthil-Nathan, S., P.G. Chung and K. Murugan, 2004. Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder *Cnaphalocrocis medinalis*. *Phytoparasitica*, 32: 433-443.
- 51. Senthil-Nathan, S., K. Kalaivani, P.G. Chung and K. Murugan, 2006. Effect of biopesticides on the lactate dehydrogenase (LDH) of the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) (Insecta: Lepidoptera: Pyralidae). *Ecotoxicol. Environ. Saf.*, 65:102-107.
- 52. Koul, O., J. Shankar and R. Kapil, 1996. The effect of neem allelochemicals on nutritional physiology of larval *Spodoptera litura*. *Entomol. Exp. Appl.*, 79: 43-50.
- 53. Koul, O., G. Singh, R. Singh and J. Multani, 2005. Bioefficacy and mode—of—action of aglaroxin A from *Aglaia elaeagnoidea* (syn. A. roxburghiana) against *Helicoverpa armigera* and *Spodoptera litura*. *Entomol. Exp. Appl.*, 114: 197-204.
- 54. Rangarajan, A., A.R. Miller and R.E. Veilleux, 2000. Leptine glycoalkaloids reduce feeding by colorado potato beetle in diploid *Solanum* sp. Hybrids. *J. Am. Sci. Hortic. Sci.*, 125: 689-93.
- 55. Bouchelta, A., A. Boughdad and A. Blenzar, 2005. Effets biocides des alcaloïdes, des saponines et des flavonoïdes extraits de *Capsicum frutescens* L. (Solanaceae) sur *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Biotechnol. Agron. Soc. Environ.*, 21-30.
- 56. Rakshit, K.D., J. Darukeshwara, K. Rathina Raj, K. Narasimhamurthy, P. Saibaba and S. Bhagya, 2008. Toxicity studies of detoxified Jatropha meal (*Jatropha curcas*) in rats. *Food Chem. Toxicol.*, 46: 3621- 3625.
- 57. Devappa, R.K., H.P.S. Makkar and R.K. Becker, 2010. Jatropha toxicity-A Review. *J. toxicol. Environ. Health*, Part B, 13:6, 476-507.
- 58. Lin, J., F. Yan, L. Tang and F. Chen, 2003. Antitumor effects of curcin from *Jatropha curcas L. Acta Pharmacol. Sin.*, 24(3): 241-246.
- 59. Millard, C.B. and R.D. Leclaire, 2008. Ricin and related toxins: review and perspective. *In:* Romano J.A., Lukey B.J. & Salem H., eds. *Chemical warfare agents: chem. Pharmacol. Toxicol. Ther.* 2nd ed. Boca Raton, FL, USA: CRC Press LLC, 423-467.
- 60. Insanu, M., C. Dimaki, R. Wilkins, J. Brooker, P. van der Linde and O. Kayser, 2012. Rational use of *Jatropha curcas* L. in food and medicine: from toxicity problems to safe applications. *Phytochem. Rev.*, 12:107-119.
- 61. Goel, G., H.P.S. Makkar, G. Francis and K. Becker, 2007. Phorbol esters: structure, biological activity, and toxicity in animals. *Int. J. Toxicol.*, 26 (4), 279-288.