Insecticidal properties of the physic nut tree
Jatropha curcas L. (Euphorbiaceae) and potential use in pest management

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General abstract

The physic nut tree *Jatropha curcas* possesses pesticidal properties and is considered a rich source of toxic phytochemicals. Our study focused on the toxic properties of *J. curcas*, in particular the seed oil (SOi), the Phorbol Esters Fraction (PEs) and the Phorbol Esters Enriched Fraction (PEEF), on different pests. The root, the leaf and the stem bark extracts were tested for their antifeedant and repellent effects. Our study concerned 3 insects´ orders: Hemiptera, Lepidoptera and Diptera. It focused on different insect species: The grain aphid *Sitobion avenae* (Hemiptera: Aphididae), the black bean aphid *Aphis fabae* (Hemiptera: Aphididae), the Diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae), the cabbage root fly *Delia radicum* (Diptera: Anthomyiidae) and the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae).

This study showed that the extracts of *J. curcas* exhibited a wide spectrum of aphicidal activities. The Phorbol esters fraction and the seed oil exhibited a high killing effect on aphids by topical and spray application too. The seed oil had an age-dependent toxicity on the black bean aphid *A. fabae*. The young nymphs were highly susceptible to the seed oil. Our work proved also the presence of a repellent effect of the extract of the primary (Tukey´s HSD; $p<0.001$) and secondary roots (Tukey´s HSD; $p<0.001$) on *S. avenae* in Petri dishes after 12 hours of application on wheat leaflets. In in vitro conditions, the secondary root extract showed a clear protective effect and provided a long lasting protection for the wheat plants (Tukey´s HSD; $p<0.001$). Our findings showed also a reproduction inhibitory effect of the seed oil on *A. fabae*. The females released on control plants gave birth to a progeny significantly larger than on plants treated with 1% of seed oil (Tukey´s HSD; $p<0.001$).

J. curcas was also investigated for its toxicity on the diamondback moth *P. xylostella*. *Jatropha* extracts induced a moderate contact toxicity on the 2nd instar larvae of *P. xylostella*. A significant difference was found between the mortality caused by the Phorbol Esters Enriched Fraction (PEEF) and the control treatment (Tukey´s HSD; $p<0.01$). The ingestion of 2% of these *Jatropha* compounds induced the mortality of 20% and 38% of the larvae respectively, for PEs and PEEF. The ingestion of PEEF induced also a deterrent effect. It affected the larval feeding behavior of *P. xylostella.* In fact, the choice test showed that the 1st and the 2nd instar larvae preferred to feed on the control leaf discs and avoided PEEF

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treated leaf discs. *Jatropha* based extracts caused also an antifeedant effect. Larvae fed for 3 days on leaf discs dipped in 0.75% PEEF had a lower weight. The leaf extract affected also the larval growth (Tukey´s HSD; p<0.001), while no effect of the stem bark extract was observed (Tukey´s HSD; p=0.83).

The ovicidal tests carried on the eggs of *D. radicum* showed a high toxicity of PEEF and seed oil. The number of eggs that failed to hatch after exposure to 1.25% and 2% of PEEF was significantly higher than the control (Tukey´s HSD; p<0.001). Moreover, a clear ovicidal effect was observed after exposure to 1.25% and 2% of SOi compared to the control (Tukey´s HSD; p<0.001). On another hand, a high toxic effect was observed after feeding, during the larval stage, on turnip pieces treated with 2% of PEEF (Tukey´s HSD; p<0.001). The ingestion of 0.5% of PEEF did not affect the pupation success. Feeding on 2% of the seed oil during the larval stage reduced significantly the pupation success (Tukey´s HSD; p>0.05). Under greenhouse conditions, we observed that drenching the soil with a concentration of 2% of PEEF reduced significantly the number of pupae in comparison with the control (Tukey´s HSD; p<0.001) on cabbage plants. The roots of cabbage plants treated with 2% of PEEF were less damaged by the feeding activity of larvae (Tukey´s HSD; p<0.001).

The topical application of the PEEF on the cuticle of the 2nd and 4th instar larvae of *H. armigera* caused a non-significant mortality. The survivorship of the 2nd instar larvae was not significantly different between treatments (Chi-square test, $\chi2 = 10.97$, d.f. = 2, p>0.05). On another hand, the feeding choice test assessed on early 1st instar larvae of *H. armigera* showed a clear deterrent effect of PEEF when mixed with the artificial diet. In this case, a significant difference was observed between treatments 24h after release of larvae in Petri dishes ($F_{2,42}=165.2$, p<0.001). At 2% concentration of PEEF mixed with the artificial medium, the larval presence on the control artificial medium was significant, whereas few released larvae were recorded on the PEEF treated diet (Tukey´s HSD; p <0.001).

Chapter 1

General introduction

Botanical insecticides have been long used as environmentally friendly alternatives to synthetic chemicals for pest management. They are renewable, degradable in the environment and relatively safe to natural enemies, non-target organisms and human beings. Plants produce a range of chemical substances to prevent attack by herbivores. Several extracts of plants have been evaluated for their activity against agriculturally important insects and being evaluated further for use in plant protection (Koul *et al.,* 2009). Such chemicals are secondary plant metabolites and include alkaloids, flavonoid and terpenoids.

Many phytocompounds showed a variety of toxic activity against pests and have been long considered as effective as synthetic chemicals for pest management (Isman *et al.,* 2005). These plant-based compounds affect arthropod pests, vectors and other infectious agents, but only few of botanicals are concretely used in agriculture (Isman *et al.,* 2000). Inspite of their biological effectiveness, the long regulatory procedure and the availability of many competing products delay their agricultural success. Few plant extracts have been developed for commercial use but many plants were screened for their toxic aspects (Isman *et al.,* 2006).

The genus *Jatropha*, rich with phytochemicals and investigated for its toxic properties, is native to tropical America and widely distributed in the tropical and subtropical areas of South America, Central America, Asia, Australia and Africa (Gübitz *et al.,* 1999). It belongs to the tribe *Jatropheae*, orders *Malpighiale* in the *Euphorbiaceae* family and contains more than 170 known species (Wurdack, 2008). The most widespread specie around the world is *Jatropha curcas* (Ratnadass *et al.,* 2012).

1.1. Origin

Although the center of origin and the center of domestication are still open questions (Dias *et al.,* 2012), this shrub is thought to be native to tropical America then widely distributed to the rest of the tropical and subtropical areas of South America, Central America, Asia, Australia and Africa (Gübitz *et al.,* 1999). Some records from antic civilizations in the Gulf of Mexico mentioned the use of *J. curcas* and its products. Also, the phenological,

biochemical and molecular studies defend this region as the center origin of this plant: many related taxa are found in this area, the rich genetic diversity and the unique phytochemical profile of the Mexican successions help to support this hypothesis (Basha *et al.,* 2009; Wilbur, 1954). In South America, *J. curcas* is found to be only associated with man settlement. In these regions, there are no records of the physic nut tree as a wild plant in forests or savannas which excludes the South American origin of this plant (Martin *et al.,* 1984).

1.2. Morphology

J. curcas is a perennial, deciduous, monoecious shrub that grows to a height of about 5 m. It has smooth bark, sturdy branches, and thick leaves. The leaves are large, shiny and glabrous, with exiguous and pilose stipules. The inflorescence is monoecious, but the individual flowers are unisexual. This plant exudes soapy and sticky latex that becomes brownish when dry. The fruit of *J. curcas* is a green subsphaeroidal capsule that becomes brown when ripe. The seeds are elliptical, black and rich in oil that can be converted into biodiesel of high quality (Ye *et al.,* 2009).

Jatropha has a succulent stem with a large water storage but low wood density. This stem water seems to play a major role in regulating the leaf water potential, serving as a buffer against low soil water potential (Maes *et al.,* 2009). The root system of this plant is composed of up to 4 lateral roots and a taproot, which can reach 5 m in depth (Foidl *et al.,* 1996; Heller, 1996; Dias *et al.,* 2007). This root system is well-developed especially in loose soil, with long and prominent taproots and lateral roots well distributed near to the soil surface.

1.3. Ecology

J. curcas is a highly climate-adapted shrub. It is present within various bioclimatic regimes from arid to rainy climate, tolerates high temperature extremes but does not prosper at low temperatures and high altitudes. It can grow on a wide range of soils provided they are well drained and aerated (Kumar *et al.,* 2008). It can be grown well on degraded soils having low fertility and moisture and also on stony, shallow and even on calcareous soils. It also develops well in sandy and gravelly soils but in heavy texture, the root development is hindered.

J. curcas is often described as a plant with low nutrient requirements. However for an economic and commercial use, this crop requires adequate fertilization (nitrogen and phosphorus) and sufficient irrigation (Brittaine *et al.,* 2010). When planted as a hedge in a poor nutrient soil, the mycorrhiza associated with the root system assimilates the micro-elements from the rhizosphere to sustain the growth and the development of the plant and to compensate the lack of fertilization (Achten *et al.,* 2008).

1.4. Uses

J. curcas has been considered as a ``miracle tree´´ (Ratnadass *et al.,* 2012) for its multipurpose use. It has been reported as a medicinal plant in many regions of the world and used for the treatment of a wide spectrum of infections. In Latin America India and also Africa, various parts of *J. curcas* are still used in ethno-medicine (Gübitz *et al.,* 1999).

Jatropha species are known for their purgative effect. The seed oil and the water extract of leaves help to cure digestive system troubles i.e. diarrhea, vomiting, retching and stomachache (Sabandar *et al.,* 2012). In Africa, the seeds are used as an anthelmintic and the leaves as a haemostatic (Watt *et al.,* 1962). The skin application of the leaf extract relieves rheumatism and inflammation symptoms. In case of pneumonia, the root decoction is drunk to clear the respiratory tracts and cure also the vermifuge (Chhabra *et al.,* 1991). The latex and stem bark are believed to be efficacious against skin infections (Heller, 1996). The oral administration of the stem bark extract is thought to enhance the excretory function of human organism (Kirtikar *et al.,* 1980; Horsten *et al.,* 1996; Goel *et al.,* 2007)

J. curcas was developed not so far as an energy resource crop and considered as a promising alternative for biofuel production (Foidl *et al.,* 1996). The seeds are rich with non-edible oil and can be converted to a high quality biodiesel. Its production is processed through the trans-esterification of the plant oil with the addition of methanol (or other alcohols) and a catalyst to end up with combustible product (Lu *et al.,* 2009). This way of use of *J. curcas* could contribute to the replacement of the fossil diesel as a major source of fuel with a significant reduction of the environmental impacts and creating socio-economic benefits for farmers (Devappa *et al.,* 2010).

Beside its medicinal and energy use, various extracts of *J. curcas* (leaves, roots, bark…) produce a wide range of biological properties such as molluscicidal, acaricidal and insecticidal activities (Ratnadass *et al.,* 2012) . These extracts have shown a toxic effect on different insect orders (Lepidoptera, Coleoptera, Diptera and Hemiptera) and offer

protection for field and stored crops. Phorbol esters and curcin are respectively the major toxic phytochemicals of the physic nut tree (Devappa *et al.,* 2011) which makes the plant non-edible by wild animals. In many tropical and subtropical countries, *Jatropha* cuttings are planted as a hedge to delimit and protect fields from wandering animals: Livestock doesn´t feed on the mature leaves and even goats will die of starvation without browsing on *Jatropha* (Henning, 2004)

In term of soil conservation, the cultivation of *J. curcas* has shown to play a major role in reducing the soil erosion by the rainwater: the robust taproot fixes the plant to the ground and protects the land from sliding while the dense biomass of the lateral roots near the surface preserves the cohesion of the soil particles and protects it from water erosion (Brittaine *et al.,* 2010). This soil conservation mechanism helps also to retain the rain water and enhances its infiltration in the soil (Achten *et al.,* 2008). *Jatropha* has also proven to be effective against wind erosion in open fields: the shape of the branches, the foliage density and the hedge biomass prevent wind erosion by breaking wind velocity and fixing the soil particles by the superficial roots (Henning, 2004).

The major interest of our study was focused on the toxic properties of the physic nut tree *J. curcas*, in particular the insecticidal potential that the extracts from this plant could exhibit on pests. The main compounds tested for their toxicity were the seed oil, the Phorbol Esters Fraction (PEs) and the Phorbol Esters Enriched Fraction (PEEF). The leaf, the root and the stem bark extract were also screened for their pesticidal potential. The killing, the antifeedant, the anti-reproduction and the deterrent effects of these compounds were investigated on agriculturally important pests.

Our study involved 3 insects´ orders including sap-sucking, leaf-chewing and root-feeding pests belonging to Hemiptera, Diptera and Lepidoptera orders. The research focused on 5 different insect species: The grain aphid *Sitobion avenae* (Hemiptera: Aphididae), the black bean aphid *Aphis fabae* (Hemiptera: Aphididae), the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae), the Diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) and the cabbage root fly *Delia radicum* (Diptera: Anthomyiidae).

The following questions were addressed in this study:

- Which compounds of *J. curcas* show an aphicidal effect? Is this effect concentration dependent?

- Does the root extract of *J. curcas* can be used as an insecticide? Does it provide an efficient protection against *S. avenae*?

-Does the seed oil effective on *A. fabae*? Does it affect the reproduction performance of this aphid when spayed on faba bean plants?

- Are phorbol esters-based compounds (PEs and PEEF) effective on *P. xylostella*? Which mode of application can induce the highest mortality?

- Does the PEEF affect the feeding behavior of the first instars larvae of *P. xylostella* and *H. armigera*?

- Are the extracts of *J. curcas* effective on *D. radicum*?

- Do the leaf and the stem bark extracts show an insecticidal effect on *P. xylostella*?

- Does the PEEF induce a pupicidal effect on *H. armigera*? Does it depend on the larval instar?

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Chapter 2

Assessment of the insecticidal effect of different compounds from the physic nut tree *Jatropha curcas* on the grain aphid *Sitobion avenae* and the black bean aphid *Aphis fabae* (Hemiptera: Aphididae)

Summary

The genus *Jatropha* is reported as a rich source of phytochemicals. The assessment of the efficacy of *Jatropha* extracts in pest control has been conducted on field and stored crops. The reported effects were ranging from repellency to feeding deterrence and mortality. In our study, we assessed the aphicidal properties of the seed oil of *Jatropha curcas* (SOi) and the Phorbol Esters (PEs) on the grain aphid *Sitobion avenae* and the black bean aphid *Aphis fabae*.

These extracts were screened for their contact and ingestion toxicity. The thoracic dorsum of young nymphs was treated with 0.5 µl of two different concentrations of the seed oil and the PEs (1% (V/V) and 2% (V/V)). In another bioassay, the contact toxicity of these extracts was assessed by spraying directly the aphids. The young nymphs were firstly released on the top of the wheat plants then sprayed 1 hour later with a fine mist spray bottle filled with the bioinsecticides. The ingestion toxicity of Phorbol Esters and the seed oil was tested by spraying the host plants until run-off with 1% (V/V) and 2% (V/V) concentrations of bioinsecticides. Also, the toxic property of the seed oil was tested on different live stages of the black bean aphid *A. fabae* (young nymphs, old nymphs and wingless adults). A dual choice test was set up to test the repellency of the root extracts of *J. curcas* on the aphid *S. avenae*. The in vitro choice test consisted of releasing in a Petri dish 15 wingless adults at a mid-distance from two wheat leaflets: one treated with 3% (W/V) of root extract and the other simply dipped in bidest water (Control). The second part of the bioassay was conducted on potted wheat plants sprayed with the same concentrations of root extract. Moreover, the systemic effect of the seed oil was tested on the black bean aphid by drenching the soil of the faba bean plants with a concentration of 1% (V/V) and 2% (V/V) of oil. The anti-reproduction properties of the seed oil were tested on treated bean plants infested with gravid females of *A. fabae* released on the treated on plants.

This study showed that the extracts of *J. curcas* exhibited a wide spectrum of aphicidal activities. These compounds exhibited a high mortality by topical and spray

application. The PEs and the seed oil showed a contact and ingestion toxicity on the grain aphid *S. avenae*. The mortality of aphids due to the topical application of 2% of PEs was significantly different compared to the control (Tukey's HSD, p<0.01). It increased after 8h to 81% and reached 91% after 24h. The spray application of 2% of PEs and 2% seed oil on infested plants caused the mortality of respectively 77% and 71% after 24h (Tukey's HSD, p<0.01). The ingestion of PEs and the seed oil had a slow toxic effect on *S. avenae*. We recorded 57% and 32% of dead aphids, respectively after ingestion of 2% of PEs and 2% seed oil (Tukey's HSD, p<0.01). This research showed also that the seed oil had an age-dependent toxicity on the black bean aphid *A. fabae*. The young nymphs were highly susceptible to the toxicity of the seed oil. In this case, a significant difference was detected in comparison with the control at 1% concentration (Tukey´s HSD; p<0.001) and 2% concentration (Tukey´s HSD; p<0.001). A significant difference was also observed between concentrations (Tukey´s HSD; p<0.001) which shows that the mortality was concentration dependent: at a concentration of 2%, the mortality rate of young nymphs reached 80% after 5 days whereas 45% were killed after the spray of 1% concentration of the seed oil.

Our work showed also, in comparison with the control, a significant repellent effect of the extract of the primary (Tukey´s HSD; p<0.001) and secondary roots (Tukey´s HSD; p<0.001) of *J. curcas* on *S. avenae* in Petri dishes after 12 hours of application. In in vitro conditions, the secondary root extract showed a clear protective effect and provided a long lasting protection for the wheat plants (Tukey´s HSD; p<0.001) . However, the primary root extract showed at a concentration of 3% a short repellent effect against aphids when applied in semi-real conditions (Tukey´s HSD; p = 0.25). The study showed also no systemic effect on the old nymphs of *A. fabae* after feeding on seed oil-treated plants: only 6.66% of the aphids were dead (Tukey´s HSD; p> 0.05). The results revealed also that the seed oil induced a very low toxicity on the adults of *A. fabae*. Only 5% of the adults were recorded dead at the end of the experiment (Tukey´s HSD; p> 0.05). Our findings showed also a reproduction inhibitory effect of the seed oil. The females released on control plants gave birth to a progeny significantly larger than on plants treated with 1% of seed oil (Tukey´s HSD; p<0.001).

1. Introduction

The family of Euphorbiaceae is one of the largest families of angiosperms, composed of over 300 genera and 8,000 species (Webster, 1994). It includes different growth forms with a predominance of trees, shrubs but also succulent plants (Devappa *et al.,* 2011). *Jatropha curcas L.* commonly referred to as ``physic nut´´, is a member of Euphorbiaceae family (Ratnadass *et al.,* 2012). This plant (a shrub or small tree) is widely distributed in tropical and subtropical areas but could be also present in extreme climates and poor growth conditions (Jing *et al.,* 2004).

J. curcas has many potential uses (Ratnadass *et al.,* 2012). It has been firstly used in ethno-medicine as a traditional medicinal plant (Openshaw, 2000). In fact, the name *Jatropha* is derived from the Greek word ''jatros'' (doctor) and ''trophe'' (food), which indicates its use for medicinal (Sabandar *et al.,* 2013) and veterinary purposes (Heller, 1996). The genus *Jatropha* is reported as a rich source of phytochemicals that can be valorized in pharmaceutical industries (Devappa *et al.,* 2010a). Based on treatments from leaves, stem, bark and roots extracts, *J. curcas* has been stated as an efficacious cure plant (Dias *et al.,* 2007) with a variety of biological activities (antitumor, cytotoxic, anti-inflammatory, antiprotozoal, anticoagulant and skin diseases) (Ratnadass *et al.,* 2012). The medicinal and pharmaceutical potential of *Jatropha* has been thoroughly researched but not fully realized.

J. curcas is also known, since few decades, as an oil seed plant (Lu *et al.,* 2009). The increasing demand for crude oil has increased the interest to this crop and its exploitation as a renewable energy source. *J. curcas* produces large seeds containing a high amount of oil (28–32%) (Devappa *et al.,* 2010b), that can be used as fuel substitute for diesel engines after transesterification (Jing *et al.,* 2004). A special interest has been also shown for the exploitation of the seed cake after oil extraction (Ye *et al.,* 2009). Since it is produced in large quantities and has a high energy value, the seed cake can be shaped in small bricks and burned as fuel (Wang, 2006) which increases the sustainability of *Jatropha* production system.

Among Euphorbiaceae, species of *Jatropha* are particularly known for their toxic properties (Ratnadass *et al.,* 2012). It is one of the richest sources of bio- compounds such as alkaloids, lignans, cyclic peptides and terpenes (Devappa *et al.,* 2011) involved in the plant defense mechanism. Various biologically active substances isolated from almost all parts of the plant have been reported to have insecticidal, acaricidal or molluscicidal activities

(Ratnadass *et al.,* 2012). The assessment of the efficacy of *Jatropha* extracts on pests has been conducted on field and stored crops. The reported effects were ranging from repellency to feeding deterrence and mortality (Silva *et al.,* 2012). The evaluation of their insecticidal effect has concerned mainly 4 insect orders with different mode of feeding (sap-sucking, leaf-chewing and stem-boring) and 20 insect families among them the family of Aphididae, investigated in our study.

The grain aphid (*Sitobion avenae*) is a serious damaging crop pest, especially in the temperate regions of the northern and southern hemispheres (Larsson, 2004). It is one of the most harmful cereal aphids in Western Europe and occurs on all cereal species including rice and maize (Llewellyn *et al.,* 2003). *S. avenae* clones have a clear preference for the ear and feeds on the rachis and on the base of the spikelets of cereals. As the ear generally remains physiologically active longer than the leaf, *S. avenae* can maintain itself longest in the crop compared to other aphids (Larsson, 2004). The damages on the yield depend on the size and duration of infestation. The typical population development consists of an initial slow build-up, rapid multiplication, slow down, stagnation and rapid decrease (Vereijken, 1979). At the absence of effective control approaches, the intensive take-up of plant nutrients causes the reduction of the number of heads and subsequently yields losses.

Another economically important pest is the black bean aphid (*Aphis fabae*) which affects a wide range of host plants. It infests beans, peas, and celery but can also attack trees and shrubs. Aphids feed on the plant by sap-sucking and produce a large amount of honeydew. This honeydew covers leaves and represents a suitable substrate for the growth of black sooty molds and other fungi, reducing photosynthesis and subsequent plant growth. This pest has a high multiplication rate which enables rapid exploitation of the host-plant. The clustering aspect of the colonies makes them visible on the buds. The high colonization potential of the different plant parts by *A. fabae* causes a stunted growth, low production and high susceptibly to diseases (Mergel, 2010).

In order to search for new botanical compounds for the control of aphids, our study focused on the insecticidal aspects of different extracts of *J. curcas* on the grain aphid *S. avenae* and the black bean aphid *A. fabae*. We investigated the following axes of research:

- Do the seed oil of *J. curcas* and the Phorbol Esters fraction (PEs) affect the survivorship of aphids? Does the contact or ingestion application cause the highest mortality rate?

- Does the aqueous root extract of *J. curcas* show protective aspects of wheat plants from *S. avenae* in in vitro conditions? Is it a long lasting effect under semi-real conditions?

- Could the systemic application of seed oil provide protection for faba bean plants from the colonization by *A. fabae*?

- Does the seed oil inhibit the reproduction performance of *A. fabae* on faba bean plants?

2. Material and methods

2.1 Host plants

Due to the variety of pest species included in our study, 2 different host plants were used to carry out the toxicity bioassays. Wheat plants (cultivar ``Dekan´´ KWS GmbH, Germany) were used as a host for the grain aphid *S. aveane*. Plants were grown in 11 cm diameter plastic pots containing a mixture of 2:1 soil : sand (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-6.3, Fertilization of 200-300mg N) and kept for 10 days in greenhouse conditions (22 ±1°C temperature, 65% relative humidity and 16L: 8D photoperiod).

Faba bean *Vicia faba* (or broad bean) plants (cultivar ``Hangdown´´ Grünkernig Gevo GmbH, Germany) were used for the bioassays on the black bean aphid *A. fabae*. Plants were grown from seeds in soil (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-6.3, Fertilization of 200-300mg N) and sand mixture (3:1 volume) was used as a growth substrate. Plants were grown under controlled conditions in a greenhouse chamber at 24 ±3°C temperature, 65% relative humidity and 16L: 8D photoperiod.

2.2. Insect culture

The aphids of *S. avenae* were collected from a laboratory rearing established from single asexual female aphids collected in Giessen (Germany) from wheat plants. Aphids were continuously reared on wheat plants grown in 11cm diameter plastic pots and covered with aerated plexiglas cylinder (30 cm × 10 cm). Aphids were transferred every 2 weeks to new wheat plants and maintained in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) under controlled conditions (20 ±1°C temperature, 70% relative humidity and 16L: 8D photoperiod) favorable for the parthenogenetic reproduction of the grain aphid.

The black bean aphid *A. fabae* was obtained from a laboratory colony reared on faba bean grown in culture trays in a wood ventilated rearing cage (90 cm × 50 cm × 50 cm). Every 2 weeks, aphids were offered a new tray of fresh faba bean seedlings after removing the old tray. The rearing tray was irrigated on alternate days and the insect culture was kept in a climate controlled room (18 ±2°C, 70 ±5% relative humidity; 16L: 8D photoperiod).

2.3. Jatropha bioinsecticides

In this study, different extracts of *J. curcas* were screened for their insecticidal effect: the seed oil (SOi), the Phorbol Esters (PEs) and the root extract (RE). The oils including SOi and PEs were provided by the company of JatroSolutions GmbH (Stuttgart, Germany) where they have been extracted and purified following the method of Devappa *et al.* (2010a).

Table 1: Composition of *Jatropha* seed oil (% m/m)

Compounds	Amounts
C12:0/ Lauric acid	<0.1
C14:0/ Myristic acid	<0.1
C16:0/ Palmitic acid	14.1
C16:1/ Palmitoleic acid	0.9
C18:0/ Stearic acid	6.1
C18:1/ Oleic acid	42.2
C18:2/ Linoleic acid	34.8
C18:3/ Linolenic acid	1.2
C20:0/ Arachidic acid	0.2
C20:1/ Gadolinium acid	0.2
C22:0/ Behenic acid	<0.1
C22:1/ Erucic acid	<0.1

(JatroSolutions GmbH, 2014)

The root extract was prepared in the laboratory before the set–up of the experiments. The root material was collected from 4 years old plants of *J. curcas,* originally from the island of Madagascar but maintained under glasshouse conditions in an environment similar to the area of origin (23 °C temperature and 65% humidity). After plant harvesting, the roots were washed and cut in small pieces then freeze dried at -20 °C. After

one week, primary and secondary roots were separately milled to a fine powder, weighed, filled in hermetic plastic bags and conserved at -20 °C for further use.

The aqueous root extract was obtained by soaking the root powder in an Erlenmeyer flask containing bidest water. The solution was kept overnight mixing on a horizontal shaker (120 rpm) (HS 250, IKA Labortechni, Germany) before being heat-treated for 1 hour in a water-bath (Köttermann GmbH & Co. KG, Uetze, Germany) at 65 °C. It was then decanted with a funnel filter paper (grade 595.5, 185 mm diameter) (Schleicher and Schuell Bioscience GmbH, Dasse, Germany) and placed in cold water to cool down in order preserve the efficacy of the phytochemicals.

2.4. Experimental bioassays

2.4.1. Toxicity of the PEs and the seed oil (SOi) on *S. avenae*

The contact toxicity of these compounds was firstly topically tested on 9 days old aphids. The thoracic dorsum of young nymphs was treated with a micropipette with 0.5 µl of different concentrations of the extracts (1% (V/V) and 2% (V/V)). TWEEN® 80 was added as emulsifier to enhance the miscibility of the compounds in water. After application, the treated nymphs were released on 7 days old wheat plants grown in 11 cm pots. In another bioassay, the contact toxicity of the seed oil and the PEs was assessed by spraying directly the aphids. The young nymphs were firstly released on the top of the wheat plants then sprayed 1 hour later with a fine mist spray bottle (2.7oz, 200 ml) containing one of the bioinsecticides.

The ingestion toxicity of the PEs and SOi was tested by spraying the host plant until run-off with a fine mist spray flask (2.7oz, 200 ml) with different concentrations of bioinsecticides (1% (V/V), 2% (V/V)). After drying, 9 days old aphids of *S. avenae* were randomly collected with a fine camel hair brush from the rearing colonies then released on the top of the treated host plants. The plants in all bioassays were covered with an aerated plexiglas cylinder (30 cm length × 10 cm diameter) and kept in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1 ∘C with a 16:8 h (L:D) photoperiod and 60% RH. Each treatment had 10 replicates and each replicate included 10 specimens. Aphid´s mortality was recorded after 3h, 8h and 24h after exposure to the extracts of *J. curcas*. Insects were considered dead when no leg or antennal movements were observed.

2.4.2. Repellent effect of the root extract on *S. avenae*

A basic dual choice test was set up to test the repellency of the root extracts on the aphid *S. avenae.* The in vitro choice test consisted of releasing 15 wingless adults at a mid-distance from a wheat leaflet treated with 3% (W/V) of root extract and a wheat leaflet simply dipped in bidest water. The bioassay was conducted in Petri dishes (140 mm diameter x 20mm height) (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany) lined with a moistened filter paper (125 mm diameter, Cat № 1001 125) (Schleicher and Schuell Bioscience GmbH, Dasse, Germany) at the bottom and sealed with parafilm after the aphid release. The Petri dishes were kept in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1 ∘C with a 16:8 h (L:D) photoperiod and 60% RH.

The second bioassay is conducted on one week old potted wheat plants. After being sprayed with a fine mist spray flask (2.7oz, 200 ml) containing 3% of root extracts, 2 wheat pots (treated and non-treated) were placed randomly in a plastic cubic cage (30 cm × 30 cm × 30 cm) separated by a distance of 10 cm. The control plants were only sprayed with bidest water. Winged aphids were released at the middle of the cage 45 mn after the plants dried. The cages were kept in a rearing room under controlled conditions (22 ± 1 ∘C, 16:8 h (L:D) and 60% RH). Each experiment was replicated 12 times in a completely random design. The number of insects was recorded in the treated and control wheat plants after 3h, 8h and 24 h.

2.4.3. Biocidal effect of the seed oil on *A. fabae*

The toxic property of the seed oil was tested on different live stages of the black bean aphid *A. fabae* (young nymphs, old nymphs and wingless adults). For that, 10 days old faba bean plants were used in this experiment. After infestation, plants were sprayed with 1% (V/V) and 2% (V/V) of a seed oil solution mixed with 0.05 % (V/V) TWEEN® 80 as emulsifier. The control plants were only treated with bidest water mixed with emulsifier. There were 10 plants each one infested with 10 aphids. The nymphs and the adults were gently collected from the rearing culture, checked for their viability and used for the plant infestation. Because of their tininess, young nymphs were enclosed in a clip cage (3.5 cm diameter x 2 cm height) on a faba bean leaf which makes them easy to monitor. For the bioassays on old nymphs and adults, aphids were released on the top of the host then the plant was covered with an aerated plexiglas cylinder (30 cm length × 10 cm diameter). The experiment was run in a glasshouse chamber (22 ± 1 ∘C, 16:8 h (L: D) and 60% RH) and the mortality was assessed

after 5 days. Aphids were considered dead if they gave no coordinated response after stimulation of the antennae and the cornicles.

2.4.4. Effect of systemic application of the seed oil on *A. fabae*

Faba bean plants were seeded in soil (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-6.3, Fertilization of 200-300mg N) and sand mixture (3:1 volume) as a growth substrate. The seedlings were grown in greenhouse conditions (22 ±1 ∘C, 16:8 h (L: D) and 60% RH) until two true leaves were fully expanded. At this stage, plants were divided into 3 different groups of 12 plants. The 1st group was twice soil drenched with a solution of SOi 1% (V/V). The 2nd group was twice soil drenched with a solution of 2% SOi (V/V). The 3rd group was daily watered with bidest water mixed with TWEEN® 80 as emulsifier. Plants in all treatments were regularly watered except in the days of soil application of the bioinsecticide. Plants were infested with 10 aphids (young nymphs or old nymphs or adults) then covered with a ventilated plexiglas cylinder and randomly placed in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1 ∘C with a 16:8 h (L:D) photoperiod and 60% RH. During the running of the bioassay, offsprings were removed from plants. After 5 days, aphid's mortality was recorded. There were 12 plants per treatment infested with 10 aphids.

2.4.5. Effect of the seed oil on the reproductive performance of *A. fabae*

In order to obtain age-synchronized nymphs, 50 wingless females were selected from the stock colony and clip caged on clean faba bean plants. After 24 hours, aphid nymphs were softly moved with a fine brush then individually clip caged on a treated host plant. Prior to infestation, plants were sprayed until run-off with different concentrations (1% (V/V), 2% (V/V) and 3% (V/V)) of seed oil before being infested with nymphs. The control plants were treated with bidest water mixed with 0.05 % (V/V) TWEEN® 80 as emulsifier. The clip caged nymphs were daily checked for their survival on the host plants until they reached the adult stage. At this step, the clip cage was removed and gravid females were left to develop, give offsprings and establish new colonies on the treated host plant. There were 12 plants per treatment covered with an aerated plexiglas cylinder (30 cm length × 10 cm diameter) and randomly distributed in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1 ∘C with a 16:8 h (L:D) photoperiod and 60% RH. After

5 days, we recorded the total number of aphids, the new formed colonies and the number of aphids per colony.

2.5. Data analysis

Statistical processing of results was carried out using the statistical software R (v 3.0.2) (R Development Core Team, 2008). Prior to analysis, all data were tested for homogeneity of variance and normality. To detect the difference between treatments, we performed One-way analysis of variance (ANOVA). If significant differences between groups were detected, means were compared by performing Tukey's test at $\alpha = 0.05$.

The mortality of aphids was analyzed using generalized linear models (GLM) taking into account the binomial distribution of the data (alive or dead). To correct data variation (overdispersion / underdispersion), quasi-binomial distribution error was used in data analyses. Thereafter, Tukey's test at $\alpha = 0.05$ was employed to detect differences between means.

Giving that some insects did not make a choice during the dual choice test, the non-choice category was also considered during the data analysis which made counts divided into 3 major groups (Control, treated and no-choice). This non-binary aspect of data deriving from choice bioassays (Control or Treated or No-choice) made it unsuitable to perform a binomial test for data analysis. In this case, we proceeded to generalized linear models (GLM) using Poisson distribution followed by quasi-poisson models analysis. In case of significant differences between groups, data were subjected to a Tukey test at $\alpha = 0.05$ to separate the means.

3. Results

3.1. Toxicity of the PEs and the seed oil *on S. avenae*

A high contact toxicity of the PEs and the seed oil was observed on the grain aphid *S. avenae*. With both compounds, aphids died soon after application of these compounds on the cuticle. The experiment indicated that the mortality was concentration-dependent. At 2% of PEs topically applied, 77% of aphids were dead after 3h of treatment whereas no aphid were dead in the control treatment (Tukey's HSD, p<0.01). The mortality of aphids due to PEs application increased after 8h to reach 81% and kept increasing to 91% after 24h. The seed oil revealed also a high aphicidal effect at 2% after 3h where 71% of aphids were dead. The mortality rate reached 79% at 24h after the topical application of the seed oil. A

concentration as low as 1% caused a mortality of 50% and 40%, respectively by the PEs and the seed oil within 24h.

The spray application of 2% of PEs and seed oil on infested plants caused the mortality of respectively 63% and 58% of aphids after 3h. After 8h, 73% of aphids were dead due to the PEs and 68% after spray with the seed oil. At the end of the bioassay, the aphicidal effect of the PEs and the seed oil was highly significant in comparison with the control (Tukey's HSD, p<0.01) and reached respectively 77% and 71% after 24h. The spray application of 1% of PEs showed a moderate effectiveness and reduced the aphid population to 49% after 24h. A lower efficacy was recorded after spray of 1% of the seed oil where only 38% of aphids were dead within 24h. No aphids were dead on the plants sprayed with water mixed with TWEEN® 80. The ingestion of PEs and the seed oil had a slow toxic effect on *S. avenae* few hours next to the release on plants. After 3h, only 5% of aphids were dead on plants treated with 2% of PEs whereas the mortality reached only 2% on wheat plants sprayed with seed oil. After 8h, the mortality rate increased and 27% of aphids were dead after ingestion of 2% of PEs and 19% after being exposed to the same concentration of the seed oil. The mortality rate increased steadily after 24h where we recorded 57% and 32% of dead aphids, respectively after ingestion of PEs and seed oil compared to the control (Tukey's HSD, p<0.01). At 1% concentration, both bioinsecticides showed a low efficacy against *S. avenae* where only 12% were dead.

3.2. Repellent effects of the root extract on *S. avenae*

In in vitro conditions, the aqueous extract of the primary roots of *J. curcas* showed a significant repellent effect overtime on the aphid *S. avenae* (Figure 1a). After 1h of release, a significant difference was observed between the different treatments ($F_{2,33}=50.43$, p<0.001) where 32.22% of aphids preferred to establish on the control wheat leaflets against 21.11% found on the treated leaflets (Tukey´s HSD; p<0.001). After 8h, we observed a slight increase of the number of aphids settled on the treated leaflets where 25% were recorded, against 37% on leaflets treated with water. Although more aphids tended to colonize the treated leaflets, a significant difference still be detected after 12h between the different treatments ($F_{2,33}= 186.54$, p<0.001). We recorded 50.55% aphids on the control leaflets of wheat against 39% on leaflets treated with the root extract (Tukey´s HSD; p<0.001).

After application, the secondary root extract showed also a clear protective effect in Petri dishes (Figure 1b). After 1h, a highly significant difference between treatments was

observed ($F_{2,33}$= 103.59, p<0.001) and 52.22% of aphids avoided the treated wheat leaflets and chose the control ones as a host (Tukey´s HSD; p<0.001). After 8h, a statistical difference was present between the different treatments ($F_{2,33}$= 112.73, p<0.001). At this time of observation, 60% of aphids were found on the control treatment while we observed a decrease of aphid's presence on the treated ones to 11.11% (Tukey´s HSD; p<0.001). At the end of the bioassay, 77.78% of aphids preferred to establish on the leaflets treated with water against 10.55% observed on the wheat treated with secondary root extract (Tukey´s HSD; p<0.001).

When sprayed on wheat plants, the primary root extract of *J. curcas* showed a significant protective effect 1 hour after spray ($F_{2,33}$= 7.31, p<0.001; Figure 1c). At this time of observation, 38.88 % of the winged adults were recorded on the control plants against 29% on the treated plants (Tukey´s HSD; p<0.001). After 8h hours of treatment, most of the adults that did not make a choice during the first hours tended to be equally distributed between the two wheat plants with 48.88% aphids recorded on the control plants against 40% on the treated plants. No significant difference was detected between the control wheat plants and the other sprayed with the root extract (Tukey´s HSD; p = 0.153). At the end of the experiment, the primary root extract showed no repellent effect against aphids when applied in semi-real conditions (Tukey´s HSD; p = 0.256) and we recorded 50.55% of aphids on the control plants against 41.66% settled on the treated plants.

The secondary root extract of *J. curcas* provided a good protection of the wheat plants after 1 hour of spray ($F_{2,33}$= 75.24, p<0.001; Figure 1d). At this time, 55% of the winged aphids chose to settle on the control plants and about 25% choose to establish on the plants treated with the secondary root extract (Tukey´s HSD; p <0.001). This repellent effect increased after 8 hours where a significant difference was observed between the different treatments ($F_{2,33}$= 94.93, p<0.001). We recorded 65% of aphids established on control wheat plants whereas only 16% were found on the wheat plants sprayed with the secondary root extract (Tukey´s HSD; p<0.001). After 12h from the beginning of the experiment, we observed more aphids established on the treated plants with about 53.33% of adults settled on the control and 32% established on the treated plants. However, a significant difference was still observed between the two treatments (Tukey´s HSD; p<0.001).

3.3. Biocidal effect of Jatropha seed oil on *A. fabae*

The seed oil had a significant influence on the survival of young nymphs of the black bean aphid *A. fabae* ($F_{2,33}$= 184.71, p<0.001). A significant difference was detected in comparison with the control at 1% concentration (Tukey´s HSD; p<0.001) and 2% concentration (Tukey´s HSD; p<0.001). A significant difference was also observed between concentrations (Tukey´s HSD; p<0.001) which showed that the mortality was concentration dependent: at a concentration of 2%, the mortality rate of young nymphs reached 80% after 5 days whereas 45% were killed after the spray of 1% concentration of the seed oil.

Although a significant difference was observed among treatments ($F_{2,33}$= 95.68, p<0.001), the seed oil of *J. curcas* showed a moderate toxicity on the old nymphs of *A. fabae*. After 5 days of exposure, the mortality induced by 1% and 2% of the seed oil reached respectively 39.16% and 56.66%. As it was the case of the young nymphs, the mortality of the old nymphs was concentration dependent and a significant difference was detected between the mortality rates induced by the two concentrations (Tukey´s HSD; p<0.001). Because of being handled, 2% of the old nymphs were dead on the control plants.

The data mortality of the adults induced by the seed oil revealed a significant difference between treatments ($F_{2,33}$= 42.17, p<0.001). In comparison with young and old nymphs, a low toxicity of the seed oil of *J. curcas* was observed on the adults of *A. fabae*. Despite a large significant difference was observed between the mortality rates at 1% and 2% (Tukey´s HSD; p<0.001), the killing effect of the oil on the adults was limited in comparison with early life stages. For example, 31.66% of aphids were dead at a concentration of 2% and only 13.33% were killed 5 days after spraying 1% of the oil. On the control plants, no adult mortality was recorded during the running time of the bioassay.

3.4. Effect of systemic application of the seed oil on *A. fabae*

Although a slight significant difference was observed between treatments ($F_{2,33}$= 27.62, p<0.05), the systemic application of the seed oil induced a low mortality on the young nymphs of *A. fabae*. The first mortality occurred 4 days after the set-up of the bioassay. After 5 days of soil application, only 10% of the aphids were dead after feeding on plants treated with 2% of seed oil. Also no significant effect of feeding on seed oil treated plants was observed on the old nymphs of *A. fabae* (Tukey´s HSD; p> 0.05) where 6.66% of the aphids were dead after 5 days. Moreover, the seed oil caused a very low toxicity on the adults *of A. fabae* and no significant difference was observed in comparison with the control

(Tukey´s HSD; p> 0.05). Only 5% of the adults were recorded dead at the end of the experiment.

3.5. Effect of seed oil on the reproductive performance of *A. fabae*

After 5 days from release, the total number of aphids differed significantly between treatments ($F_{3,44}$= 32.12, p<0.001; Figure 2). It reached 34.5 ±2.55 aphids on control plants and 24.08 ±1.16 aphids on plants sprayed with 1% of seed oil (Tukey´s HSD; p<0.001). At a concentration of 2% of seed oil, we found 16.83 ±1.30 aphids of *A. fabae* on the host plants. An important suppressive effect of the seed oil was observed on the reproduction of aphids on plants sprayed with 3% of seed oil (Tukey´s HSD; p<0.001). On these plants, only 12.5 ±0.82 aphids were found which is close to the initial number of released aphids (10 aphids).

In continuation with the previous findings, the number of offspring produced by the females was significantly different ($F_{3,44}$= 32.05, p<0.001). The females released on control plants gave birth to a progeny significantly larger than on plants treated with 1% of seed oil (Tukey´s HSD; p<0.001). After 5 days, we recorded 24.5 ±2.55 new aphids on the control faba bean plants whereas only 13 ±1.57 aphids were observed on plants sprayed with 1% of seed oil. Moreover, the reproductive performance of the females of *A. fabae* decreased as long as the concentration of the bioinsecticde increased. For example, at a concentration of 2% and 3% of seed oil were respectively recorded 6.83 ±1.30 and 2.5 ±0.82 new aphids on the sprayed plants.

According to the results of the reproduction, a significant difference was evident between treatments in term of number of new established colonies ($F_{3,44}$= 99.14, p<0.001). The results showed that among the 10 full females initially released, 9.25 ±0.21 females were able to found new viable colonies on control treatment after 2 days from release. In contrast to the control, only 5.41 ±0.48 females found growing colonies on plants sprayed with 1% seed oil whereas 3.66 ±0.37 colonies were recorded on plants treated with 2% seed oil. In comparison with the control, a high inhibitory effect on the reproduction of aphids was induced when females were exposed to a concentration of 3% of the oil of *J. curcas* (Tukey´s HSD; p<0.001). At this concentration, very few females were able to reproduce and give viable progeny. In this treatment, we recorded only 1.16 ±0.20 colonies which made females unable to colonize efficiently the host.

4. Discussion

4.1. Contact toxicity of the PEs and the seed oil *on S. avenae*

The topical and spray application of a concentration of 2% of PEs and seed oil caused a sudden death of aphids. The aphicidal effect of these compounds was fast and took few hours to induce a high mortality. According to previous studies done on *J. curcas* (Ratnadass *et al.,* 2012), the PEs and the seed oil had never been reported as neurotoxic substances to induce a similar knockdown effect. However, this could be explained by the oily texture of these extracts. As the mineral oil did, this rapid killing effect was due to the physical action of these oils more than a complex chemical reaction (Butler *et al.,* 2011). According to Stadler *et al.,* (2009), oils appeared to induce mortality due to asphyxia by obstruction of tracheae and tracheoles. This theory could be supported to explain the results of our study. The viscosity of the PEs and the seed oil played a major role in the mortality process. When sprayed or topically applied on the aphids, the oil covered the insect cuticle, blocked the spiracle and caused the suffocation of the aphid (Taverner *et al.,* 2001). The blockage of the insect tracheal system has been also stated by Richling *et al.,* (2008) on *Acheta domestica* (Orthoptera: Gryllidae); they reported a metabolism-independent mortality by accumulation of CO_2 in the insect organism after oils application.

The necrosis and cell darkening observed on the cuticle of aphids were also reported by Najar-Rodríguez *et al.,* (2007). After topical application, the lipophilic nature of oils helps to penetrate the cell, accumulate inside the cytoplasm and affect their structural and functional properties. The seed oil of *J. curcas* was also reported as a cuticle irritating compound (Sabandar *et al.,* 2013) and had been shown to cause cell disintegration and necrosis followed by a general burn effect of the insect cuticle (Devappa *et al.,* 2010b).

4.2. Ingestion toxicity of the PEs and the seed oil *on S. avenae*

As stated by Ratnadass *et al.,* (2012), our study proved that the seed oil and the PEs of *J. curcas* had high ingestion toxicity on Hemipteran species (*Myzus persicae*, *Aphis gossypii* and *Lipaphis erysimi*). The seed oil of *J. curcas* caused the mortality of aphids after feeding on wheat plant sprayed with 2% of this extract. The oil is rich with dipterpenoids which are considered to be the most important compounds synthesized by *J. curcas* (Devappa *et al.,* 2011). Also, the aphids mortality could be explained by the high amount of curcin (toxalbumin) contained in the seeds of *J. curcas*. This compound inhibits the translation

activity of proteins (or N-glycosidase activity) and could disrupt the digestive mechanism after being administrated to insects (Lin *et al.,* 2003). Moreover, the seed kernel of *J. curcas* is rich with tannins associated with toxic and anti-nutritional properties (Makkar *et al.,* 2009) causing aphids to be starved then dead.

Feeding on plants sprayed with PEs induced a higher mortality of *S. avenae* than this caused by the seed oil. After ingestion, PEs act as an analogue of diacylglycerol (DAG) and activates the proteins kinase C (Silinsky *et al.,* 2003). A normal signal transduction is triggered after the activation of the PKC by DAG (diacylglycerol), which is then rapidly hydrolyzed (Goel *et al.,* 2007). However, when introduced to organisms, the phorbol esters mimic the DAG and act independently as a stronger PKC activator that is hardly metabolized by cell. They induce subsequently a hyperactivation of PKC, initiate cell proliferation and interfere with cellular metabolic functions (Devappa *et al.,* 2010c). In our study, the mortality of aphids could be due to the ingestion of a pure concentrated PEs extract, described by Haas *et al.,* (2002) as the most toxic secondary metabolite in *J. curcas*. The insecticidal effect of PEs was already reported by Devappa *et al.* (2012) on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in in vitro bioassays.

Our results demonstrated also a lower efficacy of the ingestion bioassay than the topical application. We observed a moderate toxicity after ingestion of 2% of PEs and a low mortality after feeding on 2% of seed oil. This limited efficacy could be due to the fact that plants were not perfectly covered with the bioinsecticides. Because of this deficiency in plant spray, aphids were able to find non-sprayed areas on the host and feed safely without coming in contact with the compounds.

4.3. Repellent effect of the root extract on *S. avenae*

The root extract of *J. curcas* showed a high protective effect against the grain aphid *S. avenae*. This effect was relevant in in vitro conditions when wheat plants were sprayed with the primary and secondary root extract. This efficacy declined under semi-real conditions for the primary root extract but persisted for the secondary root extract. Based on our observations, these extracts could prevent the colonization of the plant by aphids with 2 properties: repellency and deterrence. A repellent is a volatile compound that prevents feeding by repelling the insect prior to contact with the food source. Thus, a repellent can be active at a longer distance from the food source. However, a compound that prevents or reduces feeding after the insect has already tasted the plant material is termed a deterrent

(Norris, 1986). The bioassay showed that these extracts acted as repellent for the aphids that did not come definitely in contact with treated plants and deterrent for the minority of aphids that settled temporarily on treated plants then moved to the control wheat plants. This aphid´s moving behavior is known as ``departure after landing´´ (Powell *et al.,* 2006).

These properties are due to a complex of phytochemicals known for their toxic effect. The screening of the chemical constituent of *Jatropha* plants by Devappa et *al.* (2010d) and Da Silva *et al.* (2015) lead to the isolation of alkaloids, cyclic peptides, terpenes (monoterpenes, sesquiterpenes, diterpenes and triterpenes), flavonoids, lignans, coumarins and fatty acids. In addition, El Diwani *et al.* (2009) found after HPLC analysis that the roots of *J. curcas* were rich with phenolic compounds and tannins. The major compounds were gallic, ellaguic and coumaric acids. Ellagic acid and salicylic acid were found as traces. These findings were also supported by the study of Oskoueian *et al.* (2011) on the kernel meal of *J. curcas* where HPLC analysis of the hot water extract revealed mainly the presence of gallic acid and pyrogallol as phenolic compounds, rutin and myricetin as flavonoid and daidzein (isoflavonoid). Devappa *et al.* (2011) reported the richness of the roots of *J. curcas* with diterpens and stated the presence of Caniojane, Curcusone and Jatrophol which was isolated from methylene chloride-hexane root extract of *J. curcas*.

The biological activity of most of these compounds has not been reported in previous studies. Also, the repellent and/or deterrent effect of the root extract of *J. curcas* has not been shown so far on aphids. Nevertheless, the repellent effect of *J. curcas* was already stated by Acda (2009) on the Philippine milk termite (*Coptotermes vastator*) (Isoptera: Rhinotermitidae) by a sand-barrier bioassay. The findings showed that the seed oil of *J. curcas* affected the locomotry behavior of termites which avoided tunneling in the sand chambers treated with the seed oil. In other hand, the deterrent effect of *J. curcas* was reported by Cantrell *et al.* (2011) on the mosquito *Aedes aegypti* (Diptera: Culicidae) by a mosquito biting experiment. The study showed that the triacyglycerols and the free fatty acids present in *J. curcas* induced a biting deterrent effect on *Aedes aegypti*. The gallic acid contained in the root extract of *J. curcas* would play a major role in reinforcing this deterrent property. Reese *et al.* (1976c) reported that the gallic acid affected the assimilation of food, inhibited growth, survival, pupation in the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). Moreover, the tannins predominantly present in the root extract could be involved in the plant rejection by *S. avenae* after the insertion of the stylet. Mansour *et al.*

(1997) proved a negative linear relationship between the number of piercing/sucking insects per plant (aphids, white flies and jassides) and the amount of tannins in cotton leaves. Barbehenn *et al.* (2011) concluded also that tannins are feeding deterrent to many invertebrates including sap sucking insects. In Petri dishes experiments, both extracts showed a high repellent/deterrent effect during the time of the bioassay conversely to the experiments conducted under semi-real conditions where the efficacy decreased, especially for the primary root extract. These compounds could be rapidly volatilized (repellent) but provide a residual toxic activity after spray on the plant (deterrent). This decline of efficacy in natural plant products was also reported by Zhu *et al.* (2014) after testing the antifeedancy and repellency of Catnip oil, Geraniol and an organic fatty acids mixture on horn flies *Haematobia irritans* (Diptera: Muscidae). Reifenrath *et al.* (1989) suggested mixing the extracts together instead of applying them individually to prolong their effective period.

4.4. Biocidal effect of *Jatropha* seed oil on *A. fabae*

Spraying a concentration of 2% was an effective barrier against young nymphs but less effective against old nymphs and adults of *A. fabae*. These results did not correlate with the findings on *S. avenae* where contact application of seed oil induced a high mortality of aphids. This limited efficacy on *A. fabae* could be explained by the spatial distribution of aphids on the host plant and their moving behavior from one feeding site to another.

After release on the faba bean plants, young nymphs were slow-moving or relatively immobile and tended to remain on the releasing area (Walling, 2008). Subsequently, their feeding areas were well exposed and aphids were easily targeted by spray. In this context, Northover *et al.* (2002) indicated that spraying essential oils has an effective use and good performance when pest is small in size and restricted to a small area during its lifecycle (e.g phytophagous mites and their eggs, nymphs of pear psylla, nymphs of grape leafhopper and nymphs of aphids).

However, old nymphs and adults were more mobile and left the releasing point few minutes after plant infestation. In this case, old forms were usually hidden on undersides of leaves, beneath the foliage and the growing points of the plant which made them hardly observed and did not interfere with the seed oil of *J. curcas*. Parry *et al.* (2013) stipulated that apterous (wingless) aphids move from a site to another in small distances, at a speed of 5-20 cm/mn or may run from 15-35cm/mn, depending on species. Although shorter, these

movement were thought to be enough significant for aphids to find locally a safe habitat after disturbances such as wind, pesticides, predators, crowding (Bailey *et al.,* 1995).

4.5. Effect of the systemic application of the seed oil on *A. fabae*

The plant extract used in this study failed, after systemic application, to prevent the settling of *A. fabae* on treated plants. In contrast with neem, reported as systemically taken-up by the roots of faba bean plants (Pavela *et al.,* 2004), the seed oil of *J. curcas* was not absorbed by the roots when applied to the soil. The effectiveness of the systemic application of bioinsecticides should be enhanced by the use of bioactivators, which may act as absorption enhancers and surface activators, improving penetration, translocation and effectiveness of crop protection products such as oils (Pavela, 2007). In this study, oils were used in their crude primitive form following mechanical press without any addition of bioactivators which could explain the failure to be taken-up by the roots.

4.6. Effect of the seed oil on the reproductive performance of *A. fabae*

The reproduction inhibitory effect of the seed oil, shown by our study, was already reported by Ratnadass *et al.* (2012) on *Mussidia nigrivenella* (Lepidoptera: Pyralidae), *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) and *Sitophilus granaries* (Coleoptera: Curculionidae). Host plant selection is a crucial step for aphids before starting reproducing. It is the result of a cascade of decisive behaviors guided by cues collected from the host plant (Powell *et al.,* 2006). After spraying the plant with 2% and 3% of seed oil, the fecundity of aphids was highly reduced. This effect could be explained by the richness of this extract with saturated fatty acids (palmitic acid, oleic acid, linoleic acid, myristic acid and lauric acid) which would make aphids reject the host and thus might affect the taking-up of nutritive elements needed for reproduction (Pérez-Gutiérrez *et al.,* 2011).

The oily film covering the plant surface and rich with fatty acids would inhibit nutrition, caused the starvation of insect and subsequently affect the reproduction performance of *A. fabae*. Mat Sarip *et al.* (2016) showed that in addition to its contact toxicity on *Aphis gossypii*, the lauric acid present also in the seed oil of *J. curcas*, could also affect aphid reproduction system, restrict aphid growth development and reduce aphids feeding activity. Pérez-Gutiérrez *et al.* (2011) found also that oleic, palmitic and stearic acids extracted from Papaya (*Carica papaya*) were toxic to the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) when admixed to the artificial diet of this pest and

caused the pest starvation. Moreover, Ramos-López *et al.* (2012) showed that linolenic and linoleic acids purified from the castor-oil plant (*Ricinus communis*), and also highly present in the oil of *J. curcas*, exhibited insectistatic and insecticidal activities against *S. frugiperda* and caused nutrient stress on pests.

Grüber *et al.* (1988) demonstrated that as a response to nutrient stress, during the starvation of the rose-grain aphid (*Metopolophium dirhodum*), the fat reserves content of the soma, necessary to provide lipids to the ovaries, decreased by nearly 50% and was mobilized in profit of the insect survive (Arrese *et al.,* 2010). Mat Sarip *et al.* (2016) stipulated also that the lauric acid, detected in the oil of *J. curcas*, had a similar effect to Cinnamaldehyde and Malathion and clearly affected the reproduction of *Aphis gosyypii* (Hemiptera: Aphididae). Therefore, the fecundity of aphids feeding on a plant of low nutritional quality was significantly lower. This response was expressed as a flexible reproductive strategy to nutritional stress conditions (Grüber *et al.,* 1988) and explained the anti-reproduction effect of the extracts of *J. curcas*.

5. Conclusion

This study demonstrated that the extracts of *J. curcas* exhibited a wide spectrum of aphicidal activities. Our research proved the killing, repellent, antifeedant and anti-reproduction effect of the extracts of *J. curcas*. The PEs and the seed oil showed a contact and ingestion toxicity on the grain aphid *S. avenae*. These compounds exhibited a high mortality by topical and spray application caused by disruption of respiratory functions. When sprayed or topically applied on the aphids, the oil covered the insect cuticle, blocked the spiracle and caused the suffocation of aphid. Some necroses were also developed. After topical application, the lipophilic nature of oils helps to penetrate the cell, accumulate inside the cytoplasm and affect their structural and functional properties.

This research also showed that the seed oil had an age-dependent toxicity on the black bean aphid *A. fabae*. The young nymphs were more susceptible to the seed oil then the old and adults. The spatial distribution of aphids on the host plant and their moving behavior made the young immobile nymphs easily targeted than the mobile old stages by spraying the seed oil. Our study showed also a reproduction inhibitory effect of the seed oil. The oily film covering the plant surface and rich with fatty acids would inhibit nutrition, caused the starvation of insect and subsequently affect the reproduction performance of *A. fabae*.

The study showed also that the root extract of *J. curcas* induced a repellent effect on the grain aphid *S. avenae* resulting in a high protective effect against this aphid. This effect was relevant in in vitro conditions when wheat plants were sprayed with the primary and secondary root extract. The bioassay showed that these extracts would act as repellent for the aphids that did not come definitely in contact with treated plants and deterrent for the minority of aphids that settled temporarily on treated plants then moved to the control wheat plants.

This study revealed the insecticidal properties of *J. curcas*. The seed oil and the PEs extracted from this plant could be promising alternatives to synthetic chemicals for the control of aphids. The efficacy of these compounds could be valorized in crop protection and enhanced by combination with other safe plant protection agents such as entomopathogenic fungi. The repellent properties of the root extract could be improved by the elucidation and purification of the major compounds involved in this effect.

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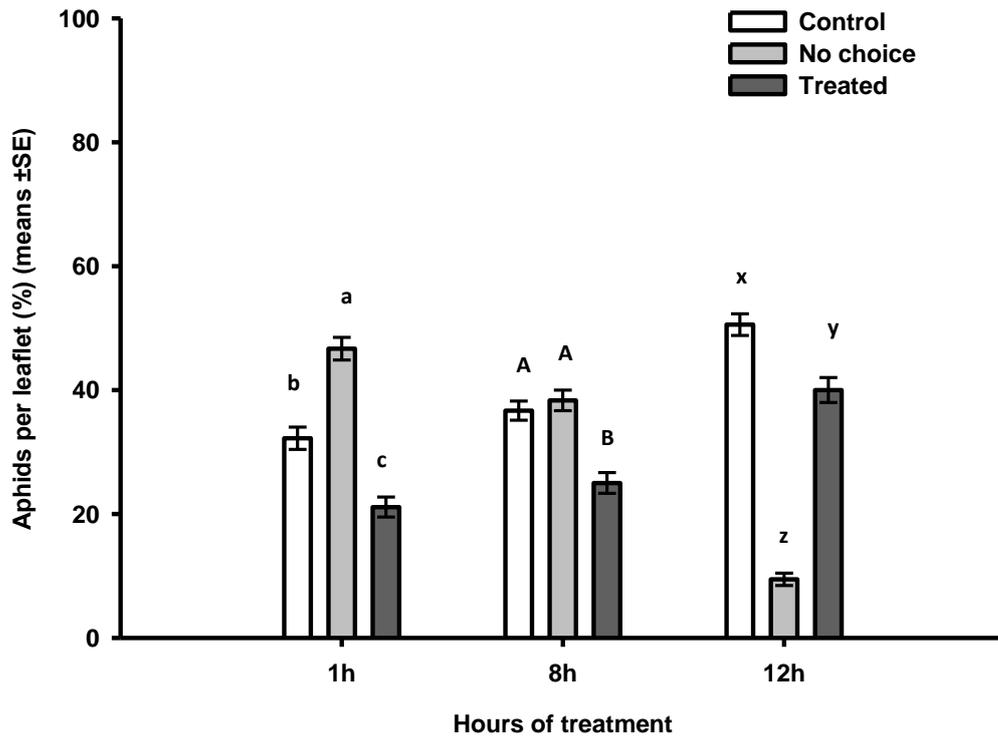
Figure 1a: Repellent effect of the primary root extract of *J. curcas* on the grain aphid *S. avenae* in in vitro conditions. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
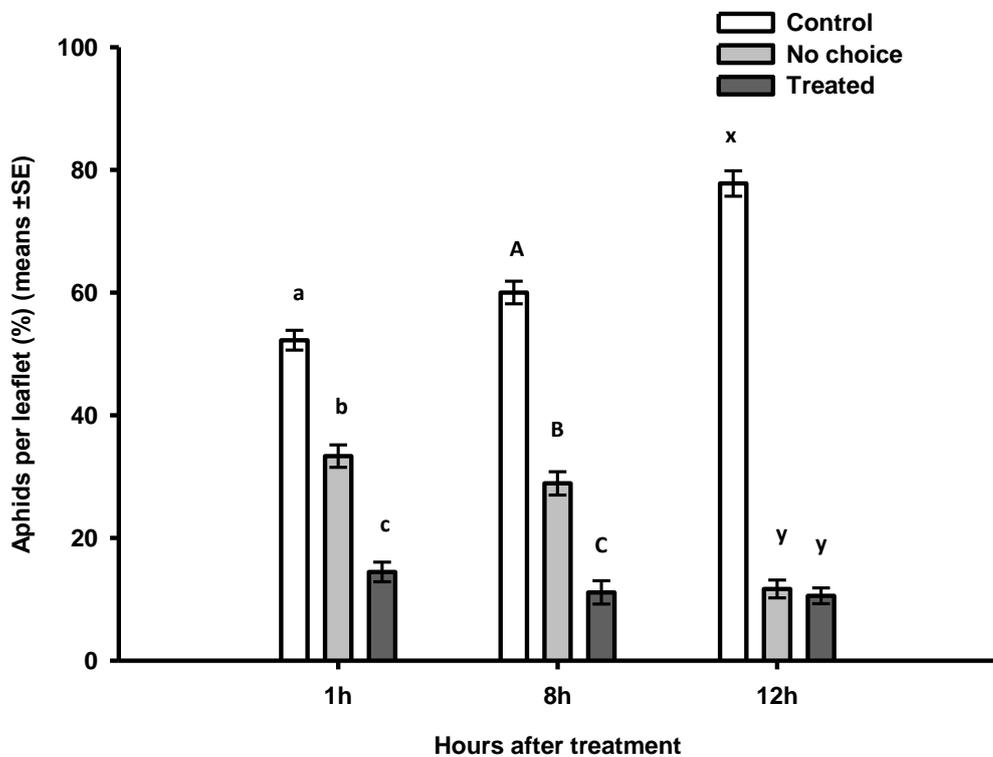
Figure 1b: Repellent effect of the secondary root extract of *J. curcas* on the grain aphid *S. avenae* in in vitro conditions. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
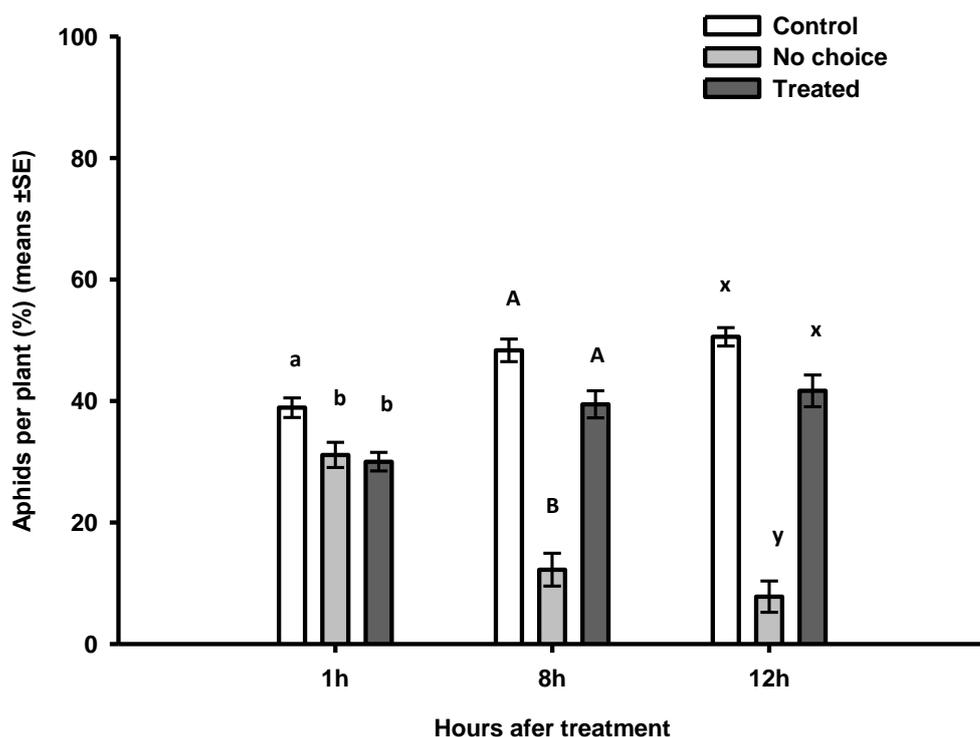
Figure 1c: Repellent effect of the primary root extract of *J. curcas* on the grain aphid *S. avenae* on wheat plants. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
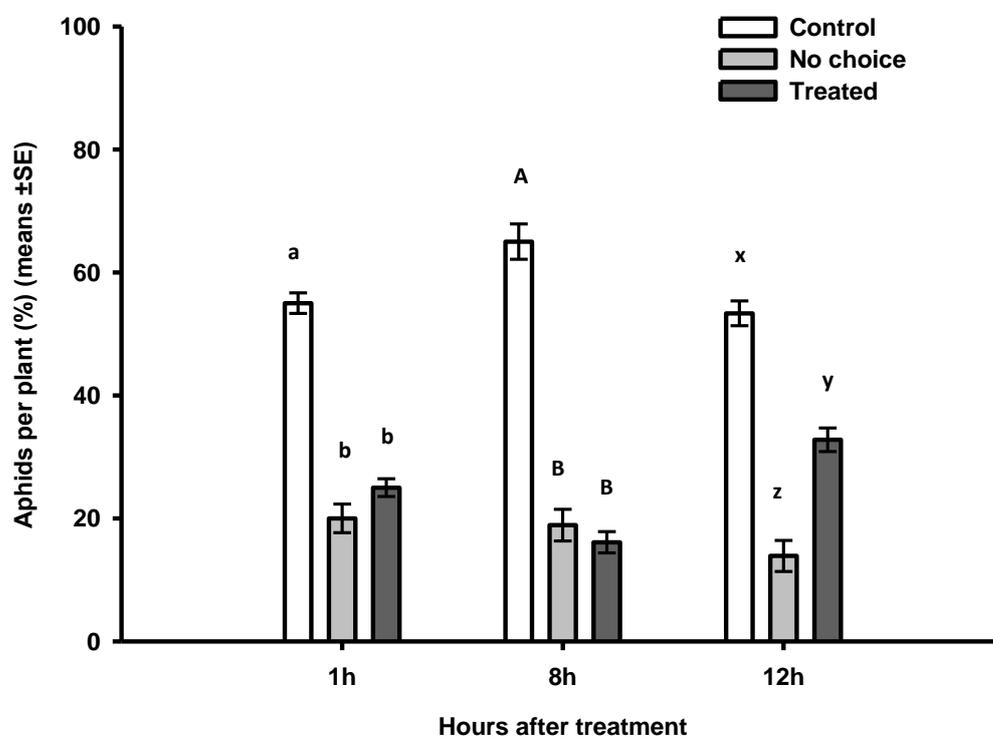
Figure 1d: Repellent effect of the secondary root extract of *J. curcas* on the grain aphid *S. avenae* on wheat plants. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
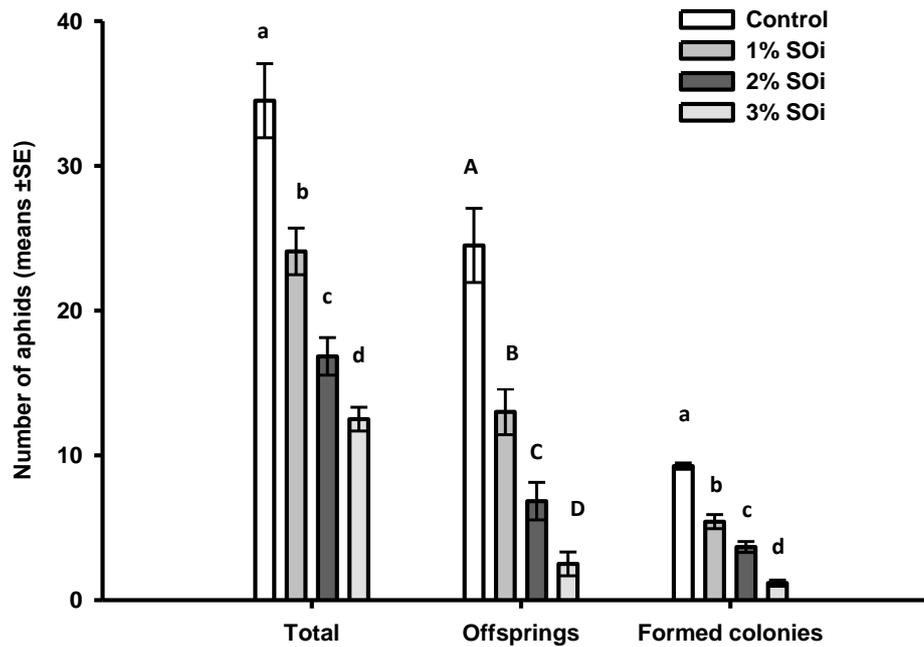
Figure 2: Anti-reproduction effect of the seed oil (SOi) of *J. curcas* on the black bean aphid *A. fabae*. The **Total** included the number of the females initially released and their offsprings; **Offsprings** showed the number of nymphs produced by the surviving females on host plants and **Formed colonies** expressed the number of colonies established by the surviving females. The data derived from each measured parameter (total, offsprings and formed colonies) were independently analyzed and statistically compared within the same parameter. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, $p < 0.05$).

Chapter 3

Study of the killing and antifeedant effects of some botanical extracts from the physic nut tree *Jatropha curcas* on the Diamondback moth (DBM) *Plutella xylostella* (Lepidoptera: Plutellidae)

Summary

The physic nut tree *Jatropha curcas* was investigated for its toxicity on the diamondback moth *Plutella xylostella*. The study showed that the Phorbol Esters Enriched Fraction (PEEF) and the Phorbol Esters (PEs) induced a moderate toxicity when topically applied on the early 2nd instar larvae of *P. xylostella*. After 4 days, a significant difference was found between the mortality caused by the PEEF and the control treatment (Tukey´s HSD; p<0.01) while the PEs showed a non-significant contact toxicity (Tukey´s HSD; p =0.27). At the end of the bioassay, 18% of larvae were recorded dead after being treated with PEs and 26% of larvae were killed after exposure during 6 days to PEEF.

The ingestion of 2% of PEs induced the mortality of 16% of the larvae and 28 % after feeding for 48h on PEEF treated discs. After 4 days, the killing effect of the compounds increased slightly to 20% and 38% respectively for PEs and PEEF. In this case, a significant difference was evident for the PEs (Tukey´s HSD; p<0.001) and a highly significant for the PEEF (Tukey´s HSD; p<0.001) compared to the control. After 6 days, more larvae of *P. xylostella* were dead and a significant difference was observed between treatments ($F_{2,27}$= 27.44, p<0.001). After 6 days, the killing effect of the compounds increased slightly to 20% and 38%, respectively for PEs and PEEF.

The PEEF affected also the larval feeding behavior of *P. xylostella*. After 12h, the 1st instar larvae of *P. xylostella* preferred to feed on the control cabbage leaf discs and avoided PEEF where only 10.55% of larvae were recorded (Tukey´s HSD; p<0.001). The 2nd instar of *P. xylostella* showed also avoidance to PEEF. After 12h, the larval presence increased steadily to reach 69.44% on the control leaf discs (Tukey´s HSD; p<0.001). In contrast to the early larval stages, the 3rd instar larvae of *P. xylostella* did not show any preference to any treatment during the bioassay (Tukey´s HSD; p=0.70). Larvae fed freely on both leaves without making a clear choice: 46.66% larvae were recorded on the control discs while 41.11% observed on PEEF treated leaves.

Jatropha based extracts affected the larval weight of *P. xylostella* ($F_{3,44}$=16.14, p<0.001). After 3 days, larvae fed on leaf discs dipped in 0.75% PEEF had a lower weight and leaves were less consumed. The leaf extract affected also the larval growth and showed also a protective effect (Tukey´s HSD; p<0.001), while no effect of the stem bark extract was observed (Tukey´s HSD; p=0.83). As a consequence for being underfed, the pupal weight of *P. xylostella* was slightly affected ($F_{3,44}$=4.90, p<0.05). Under greenhouse conditions, *Jatropha* extracts did not show the same efficacy as it was observed under laboratory conditions. In fact, no effect of the leaf extract on the weight was observed (Tukey´s HSD; p=0.35). However, the suppressive effect of PEEF on the body weight was still observed after 5 days of feeding. The PEEF induced the highest protective effect for the leaf surface (Tukey´s HSD; p<0.05). However, the stem bark extract did not show any significant protection in greenhouse conditions (Tukey´s HSD; p=0.94).

Keywords: *Jatropha curcas*, *Plutella xylostella,* Phorbol Esters fraction, Phorbol Esters Enriched Fraction, antifeedant, larval growth.

1. Introduction

Botanical insecticides have been long used as environmentally friendly alternatives to synthetic chemicals for pest management. Plants produce a range of chemical substances to prevent attack by herbivores (Ohmura *et al.,* 2000; Barbehenn et *al.,* 2009; Dowd *et al.,* 2011). Several extracts of plants have been evaluated for their activity against agriculturally important insects and being evaluated further for use in plant protection (Koul *et al.,* 2008).

The genus *Jatropha*, rich with phytochemicals and investigated for its toxic properties, is native to tropical America and widely distributed in the tropical and subtropical areas of South America, Central America, Asia, Australia and Africa (Gübitz *et al.,* 1999). It belongs to the tribe *Jatropheae*, orders *Malpighiale* in the *Euphorbiaceae* family and contains more than 170 known species (Wurdack, 2008). The most widespread specie around the world is *Jatropha curcas* (Ratnadass *et al.,* 2012), assessed in our study for its toxicity on the diamondback moth *Plutella xylostella*.

The diamondback moth *Plutella xylostella* (DBM) is a serious pest of Brassica (Talekar *et al.,* 1993; Sarfraz *et al.,* 2005) and causes high losses on cruciferous vegetables (e.g. cabbages, broccoli and cauliflowers) and oilseed crops (e.g. canola and mustard) (Furlong *et al.,* 2013). This pest is believed to be the most universally distributed of all Lepidoptera (Shelton, 2004). However, the high diversity of its natural enemies and the large host complex recorded in South Africa supported this area as the center of origin of this pest and its spread later on with the host plant all over the world (Kfir, 1998). Nonetheless, the study of the genetic traits among various populations of *P. xyllostella* in different parts of the world (South Africa, France, Japan, Australia…) have shown large genetic variations, which makes it difficult to fix this pest to a precise geographic origin (Pichon *et al.,* 2006). *P. xylostella* has a wide ecological plasticity, which allows it to develop in various climatic conditions. The moths overwinter as adults among field debris of cruciferous crops, and are still active during warm winter episodes (15°C-18°C). In warm conditions, the lifecycle including 4 larval stages takes about 3 weeks and the adults can complete 18 generations on Brassica.

P. xylostella is an oligophagous pest, which feeds only on plants of the family Brassicaceae. It damages the leaves of its host plants from the seedlings until harvest period, but only the larval stage is herbivorous (Amit *et al.,* 2001). The larva is a voracious surface feeder but does not destroy the veins. With its chewing mouthparts, the larva feeds on the

undersides of leaves leaving a translucent epidermis. As the leaf grows, the damaged surface rips creating holes and destroying the leaf. The larvae can also nibble the chlorophyll-rich green areas of stems and pods (Cabi, 2016) and hamper the plant development. The damage is often first evident on plants growing on ridges and knolls in the field (Canola Council of Canada, 2014). When the plant is seriously infested, the larval population may heavily destroy the foliar tissue and disrupt the head formation in cabbage, broccoli, and cauliflower. Also, many caterpillars or cocoons are sometimes hidden in the heads, and these may be rejected for commercialization.

Although the control of the diamondback moth is mainly based on chemicals (Canola Council of Canada, 2014), the use of bioinsecticides occupies a major place in management programs against this pest (Liang *et al.* (2003); Nadeem *et al.* (2012)). The purpose of this study was to assess the toxicity of different extracts from the physic nut tree *J. curcas on P. xylostella.* The Phorbol Esters fraction (PEs), the Phorbol Esters Enriched Fraction (PEEF), the seed oil, the aqueous leaf extract, the stem bark and the root extracts were screened for their toxic effects on this pest. The insecticidal properties of these compounds were compared with standard bioinsecticides such as NeemAzal and Spinosad, known as effective formulations to control the populations of the diamondback.

We addressed the following questions:

- Does the topical application of the Phorbol Esters fraction (PEs) induce the mortality of the larvae of *P. xylostella*?

- Is the Phorbol Esters Enriched Fraction (PEEF) highly toxic to larvae than PEs?

- Can the ingestion of PEs or PEEF affect the survivorship of larvae?

- Does PEEF affect the larval feeding behavior of *P. xylostella*? Does this effect depend on the larval instar stage?

- Do the stem bark and the leaf extract induce an antifeedant effect on the 2nd instar larvae?

2. Material and methods

2.1. Host plants

Cabbage plants *Brassica oleracea* var. *capitata* L. (Cultivar: ``Furchenkohl´´; Norddeutsche Pflanzenzucht Hans-Georg Lembke KG) were used as host for the toxicity bioassays on *P. xylostella*. The cabbage plants were grown in plastic trays from seeds in a

greenhouse chamber (22 ±3 °C; 80% relative humidity; 16L: 8D photoperiod). After 10 days, seedlings were transplanted into 11 cm diameter pots filled with non-sterile soil (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-6.3, Fertilization of 200-300mg N) and sand mixture (3:1 volume). Plants were grown in the chamber, regularly irrigated and fertilized once a week (fertilizer: Hakaphos 2g/L (15% N, 11% P2O5, 15% K2O, 1% Mg, 0.1% Fe, 0.1% Mn, 0.04% Cu, 0.025 %B and 0.005 % Mo)).

2.2. Insect culture

Larvae and adults of DBM were obtained from a laboratory culture maintained in a wood cage (90 cm × 50 cm × 50 cm) in a climate controlled room (21 ±1°C temperature, 60 ± 5% relative humidity and L16:D8 photoperiod) and reared on fresh cabbage plants (cultivar: Furchenkohl). Adults were provided weekly with 2 weeks-old fresh cabbage plants as oviposition support and food resource for the larvae. Old plants were removed from the cage to avoid the contamination of the rearing by potential saprophyte pathogens such as fungus and some species of spider mites.

2.3. Jatropha bioinsecticides

In this study, many different extracts of *J. curcas* were screened for their insecticidal effect: the seed oil (SOi), the Phorbol Ester (PEs), the Phorbol Ester Enriched Fraction (PEEF), the leaf extract and the stem bark extract. The oils were provided by the company of JatroSolutions GmbH (Stuttgart, Germany) where they have been extracted and purified following the method of Devappa *et al.* (2010a) then stocked in a cold room to be used in bioassays. The other botanical extracts of *J. curcas* were prepared from greenhouse - cultivated plants.

The leaf and stem bark based extracts were prepared in laboratory. The plant material was collected from vigorous plants of *J. curcas* grown under glasshouse conditions (temperature and 65% humidity 23º C) in an environment similar to the area of origin (Madagascar). Plants were gently harvested and the sand was removed with water pressure until roots were completely clean. Fully grown green leaves were cut from the upper part of the plants. We used only young leaves whereas old leaves were discarded. The stem was firstly cleaned with a moistened tissue to remove the dust; then the bark was gently shelled from the middle part of the stem. After being cleaned, the leaves and the stem bark of *J. curcas* were cut in small pieces and freeze dried at -20 º C. After one week, the plant

material was separately milled to a fine powder, weighed, filled in hermetic plastic bags and conserved at -20 º C for further use. The aqueous leaf and stem bark extracts were obtained by soaking the plant powder in an Erlenmeyer flask containing bidest water. The solution was kept overnight mixing on a horizontal shaker (120 rpm) (HS 250, IKA Labortechni, Germany) before being heat-treated for 1 hour in a water-bath (Köttermann GmbH & Co. KG, Uetze, Germany) at 65 ◦C. It was then decanted with a funnel filter paper (grade 595.5, 185 mm diameter) (Schleicher and Schuell Bioscience GmbH, Dasse, Germany) and placed in cold room for further use.

2.4. Experimental bioassays

2.4.1. Contact toxicity of PEs and PEEF

The contact toxicity of *Jatropha* compounds was tested on 2nd instar larvae of *P. xylostella* collected from a stock colony reared in laboratory conditions (21 ±1°C temperature, 60% relative humidity and L16:D8 photoperiod). The methodology consisted of topically treating the larvae by applying 0.5 µl of different concentrations of the compounds on the anterior pronotum using a micro-pipette. The bioassay was composed of 3 different treatments: the control group with larvae simply treated with water mixed with 0.05 % (V/V) TWEEN® 80, the Phorbol Esters 2% (V/V) (PEs) and the Phorbol Ester Enriched Fraction (PEEF) 2% (V/V). The treated larvae were then released per group of 5 larvae on 3 weeks old non-treated cabbage plants to feed freely on young leaves. The bioassay was conducted under greenhouse conditions (22 ±3°C temperature, 60% relative humidity and 16L: 8D photoperiod) until the larvae pupated. The larval mortality was checked at 2 days, 4 days and 6 days after treatment. Larvae were considered dead when no reaction/movement to the stimulation with a blunt needle was observed. At the end of the experiment, the pupae were gently collected with forceps and kept on a filter paper in white boxes (18cm length ×13cm width ×5.5cm height) until emergence in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) with the same conditions of the greenhouse. There were 10 cabbage plants per treatment infested each with 5 larvae of *Plutella*.

2.4.2. Ingestion toxicity of PEs and PEEF

The ingestion toxicity of the PEs and PEEF was tested on 3 days old larvae of *P. xylostella*. Newly hatching larvae were gently collected with a fine camel brush from the stock colony; then transferred to feed for 3 days on clean cabbage plants before use in the

bioassay. TWEEN® 80 was used as emulsifier. The experiment was set-up with 3 different treatments in a greenhouse cabinet (22 ±3°C temperature, 60% relative humidity and 16L: 8D photoperiod): control plants treated with water, plants treated with PEs and plants treated with PEEF. The plants were sprayed until run-off with 2% (V/V) of the extracts then left for 1 hour to completely dry. In order to avoid feeding pressure on the host, each plant was infested with only 5 larvae of *P. xylostella* feeding freely on cabbage leaves until pupation. Pupae were collected and kept until emergence in a climatic chamber, the same as in the previous bioassay.

2.4.3. Effect of the PEEF on the feeding behavior of the 1st, 2nd and 3rd instar larvae

In this bioassay, the larvae of *P. xylostella* were exposed in vitro to a feeding choice test. Leaf discs of 4 cm diameter were cut with a clean leaf borer from 3 weeks old cabbage plants reared in greenhouse conditions. In this dual choice test, the control leaf discs were immersed for 1 mn in a solution of bidest water, whereas the treated leaf discs were immersed in a solution of 2% (V/V) of PEEF contained in a beaker of 200 ml. TWEEN® 80 was used in both treatments as emulsifier. Leaf discs were left to dry under laboratory conditions on a clean surface then placed with forceps separated by 5 cm in a 14 cm Petri dish on a moistened filter paper (125 mm diameter, Cat № 1001 125) (Schleicher and Schuell Bioscience GmbH, Dasse, Germany). 15 healthy larvae of *Plutella* (1st or 2nd or 3rd instar) were introduced in each Petri dish at 6 cm away from the leaf discs. The larvae were left to make a choice and the Petri dishes were sealed with parafilm to avoid dryness of the leaf discs. Larvae were counted on each disc at 1h, 8h and 12h after release. The Petri dishes (12 per instar larvae) were randomly distributed and kept in a climatic chamber under controlled conditions (22 ±1 ◦C, 16:8 h (L: D) and 60% RH).

2.4.4. Anti-feedant effect of the PEEF, the stem bark extract and the leaf extract

In this experiment, the antifeedant properties of the PEEF, the stem bark (BR$_{ex}$) and the leaf extract (Lf$_{ex}$) were firstly studied under in vitro conditions in Petri dishes (86.5 mm diameter x 14.5 mm height) (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany). In this part, the same methodology as in the previous bioassay was followed to treat the cabbage leaf discs. The experiment had 4 different treatments: the control leaf discs, the leaf discs treated with 0.75% (V/V) of PEEF, the leaf discs treated with 2% of BR$_{ex}$ (W/V) and the discs treated with 2% Lf$_{ex}$ (W/V). The 2nd instar larvae were firstly weighed on a microbalance

(Sartorius MC5 ((-0CE), SC2 (-0CE), Max 5.1 g), Göttingen, Germany) then individually introduced in Petri dishes where leaf discs were already placed.

The second bioassay was conducted under greenhouse conditions (22 ±3°C temperature, 80% relative humidity and 16L: 8D photoperiod) on 3 weeks old cabbage plants. The same concentrations of PEEF, BR_{ex} and Lf_{ex} were tested for their effectiveness under semi-real conditions. For that, one young cabbage leaf was gently treated with the bioinsecticide solution then left to dry for 1 hour. After that, one newly molted 2nd instar larva of *Plutella* was clip-caged (5 cm diameter × 1 cm height) on the abaxial face of the plant. In laboratory and greenhouse experiments, larvae were left feeding freely on the leaf surface for 4 days. At the end of the 4th day larvae were weighed, leaves were labeled and larvae were individually transferred to new treated leaves where they fed until pupation. At the end of the bioassay, the consumed leaf area was assessed by the Digi-trace program for image analysis (IMATEC Elektronische Bildanalyse Systeme GmbH) and pupae were weighed. There were 12 replicates per treatments in a completely random design.

2.5. Data analysis

Statistical processing of results was carried out by standard methods using the statistical software R (v 3.0.2) (R Development Core Team, 2008). Prior to analysis, all data were tested for homogeneity of variances and normality. To detect the difference between treatments, we performed One-way analysis of variance (ANOVA). If significant differences between groups were detected, means were compared by performing Tukey's test at $\alpha = 0.05$. The mortality of larvae after treatment with the extracts of *J. curcas* was analyzed using generalized linear models (GLM) taking into account the binomial distribution of the data (alive or dead). To correct the anomalies in data variation (overdispersion / underdispersion), we proceeded to the quasi-binomial distribution error in data analysis. Thereafter, Tukey's test at $\alpha = 0.05$ was employed to detect differences between means.

Giving that some larvae did not make a choice during the feeding choice test, the non-choice category was also considered during the data analysis which made counts divided into 3 major groups (Control, treated and no-choice). This non-binary aspect of data deriving from choice bioassays (Control, Treated or No-choice) made it unsuitable to perform a binomial test for data analysis. In this case, we proceeded to generalized linear models (GLM) using Poisson distribution followed by quasi-poisson models analysis in case

of dispersion anomalies. In case of significant differences between groups, data were subject to a Tukey's test at α = 0.05 to separate the means.

3. Results

3.1. Contact toxicity of the PEs and PEEF

Jatropha based extracts induced a moderate toxicity when topically applied on the early 2nd instar larvae of *P. xylostella* (Figure 1). After 2 days of application, the PEs and PEEF induced a low mortality rate which reached respectively 6% and 14%. After 4 days, a significant difference was found between the mortality caused by the PEEF and the control treatment (Tukey´s HSD; p<0.01) while the PEs showed a non-relevant contact toxicity (Tukey´s HSD; p =0.27). After 6 days, we observed an increase of the larval mortality rate and a significant difference was observed between treatments ($F_{2,27}$=12.60, p<0.001). At the end of the bioassay, 18% of larvae were recorded dead after being treated with PEs and 26% of larvae were killed after exposure during 6 days to PEEF. After this time, no mortality was recorded. All the surviving larvae in all treatments continued their development to reach the pupal stage. All pupae hatched and gave healthy flying adults. We observed no larval mortality in the control groups.

3.2. Ingestion toxicity of PEs and PEEF

We found a significant difference after 2 days of feeding on cabbage leaf discs ($F_{2,27}$=12.34, p<0.001; Figure 2). In comparison with the control, the ingestion of 2% of PEs induced the mortality of 16% of the larvae (Tukey´s HSD; p<0.05) and a highly significant larval mortality which reached 28 % after feeding for 48h on PEEF treated leaf discs (Tukey´s HSD; p<0.001). After 4 days, the killing effect of the compounds increased slightly to 20% and 38% respectively for PEs and PEEF. In this case, a significant difference still evident for the PEs (Tukey´s HSD; p<0.001) and a highly significant for the PEEF (Tukey´s HSD; p<0.001) compared to the control. After 6 days, more larvae of *P. xylostella* were dead and a significant difference was observed between treatments ($F_{2,27}$= 27.44, p<0.001). The overall mortality after ingestion of the PEs and PEEF reached 28% and 38%, respectively, much higher than the mortality rate induced by contact toxicity. After incubation, all surviving pupae hatched and gave healthy adults. There was no mortality of larvae fed on control leaf discs.

3.3. Effect of the PEEF on the feeding behavior of the 1[st,] 2nd and 3rd instar larvae

In this choice test, a significant difference was observed between treatments few hours after the set-up of the experiment ($F_{2,33}$=126.79, p<0.001; Figure 3a). At 1 hour after release, 55% of the 1st instar larvae chose to feed on the control leaf discs. Only 10.5 % of the larvae were found on the leaf discs treated with PEEF. These results were significantly different from the control (Tukey´s HSD; p<0.001). After 8 hours, more larvae moved from the PEEF treated leaf discs to feed on the control leaves which led to the decrease of the larval presence on PEEF discs to 11.11%. After 12h, the 1st instar larvae of *P. xylostella* showed a clear feeding preference to the control leaf discs simply dipped in water (Tukey´s HSD; p<0.001) and only 10.55% of larvae were recorded in the PEEF treatment.

The second instar of *P. xylostella* significantly preferred the control leaf discs after 1h compared to the PEEF treated leaves (Tukey´s HSD; p<0.001; Figure 3b). We recorded 44.44% larvae feeding on control leaf discs against 24.5% present on the leaves dipped in PEEF. After 8h, this difference between treatments became larger ($F_{2,33}$=109.96, p<0.001), where 50% of larvae avoided settling on the PEEF treated discs and chose to feed on the control leaves (Tukey´s HSD; p<0.001). Only 13.88% were observed on PEEF based treatment. After 12h, the larval presence increased steadily to reach 69.44% on the control leaf discs and showed consequently a highly significant difference in comparison with the PEEF treated discs (Tukey´s HSD; p<0.001). We observed also a small increase of the larval number on bioinsecticide treated discs to 18%.

In contrast to the early larval stages, the 3rd instar larvae of *P. xylostella* did not show any preference to any treatment during the bioassay (Figure 3c). After 1 hour, 31.66% of 3rd instar larvae were feeding on the control cabbage discs while about 30% on the discs dipped in PEEF. There was no statistical difference between the two treatments (Tukey´s HSD; p=0.70). After 8h, the PEEF leaf application did not affect the feeding behavior of larvae. The ones that did not make a choice during the previous hours tended to be homogenously distributed between the two treatments. Subsequently, no significant difference was observed (Tukey´s HSD; p=0.06). After 12h, the 3rd instar larvae of *P. xylostella* fed freely on both treatments without showing any feeding preference: 46.66% larvae were recorded on the control discs while 41.11% observed on PEEF treated leaves. There was no statistical difference between the two treatments (Tukey´s HSD; p=0.39).

3.4. Anti-feedant effect of the PEEF, the stem bark extract and the leaf extract

Under in vitro conditions, *Jatropha*-based extracts affected significantly the larval weight of *P. xylostella* after feeding on cabbage leaves ($F_{3,44}$=4.84, p<0.05). In comparison with the control, larvae fed on leaf discs dipped in 0.75% PEEF had a significantly lower weight than the control (Tukey's HSD; p<0.05). The leaf extract applied on cabbage leaves affected also the weight of larvae after feeding (Tukey's HSD; p<0.05), while no effect of the stem bark extract was observed on larval growth (Tukey's HSD; p>0.05). After 4 days of feeding, the highest larval weight was observed in the control treatment where larvae reached a weight of 4.12 ±0.09 mg. The PEEF and the leaf extract treatments showed a clear suppressive effect on larvae which weighed respectively 3.53 ±0.13 mg and 3.57 ±0.12 mg.

As a consequence for being underfed, the pupal weight of *P. xylostella* was slightly affected. It was significantly different among treatments ($F_{3,44}$=4.90, p<0.01). We observed a significant difference of the pupal weight after feeding on 0.75% of PEEF (Tukey's HSD; p<0.05) and 2% of leaf extract (Tukey's HSD; p<0.05) compared to the control. The stem bark showed no significant effect on the pupal weight (Tukey's HSD; p>0.05). In in vitro conditions, the leaf area consumed during 4 days was significantly different between treatments ($F_{3,44}$=16.75, p<0.001). The cabbage leaf discs treated with PEEF were less consumed than the control leaves (Tukey's HSD; p<0.001). The leaf extract showed also a significant protective effect when applied on the leaf surface in comparison with the control (Tukey's HSD; p<0.05). In this case larvae consumed 3.92 ±0.12 cm^2 while 4.51 ±0.09 cm^2 of the leaf area was damaged in the control treatment. The stem bark extract of *J. curcas* did not show any effect on the feeding voracity of *P. xylostella*. The larvae consumed 4.64 ±0.14 cm^2 of the leaf area which was not significantly different from the control (Tukey's HSD; p=0.91).

Under greenhouse conditions, *Jatropha*-based extracts did not show the same efficacy as it was observed under laboratory conditions. Despite the statistical difference between treatments after 4 days of feeding ($F_{3,44}$=3.03, p<0.05), no effect was observed on the larval weight after feeding on plants treated with the leaf extract (Tukey's HSD; p>0.05). After 4 days of feeding, the larval weight reached 4.22 ±0.06 mg for larvae fed on leaf extract treated plants and 4.30 ±0.12 mg for larvae fed on control cabbage plants. A statistical difference was however evident of the weight of larvae fed on PEEF treated plants (Tukey's

HSD; p<0.05), while no significant difference was observed for the stem bark treatment (Tukey´s HSD; p>0.05).

Consequently, the pupal weight of *P. xylostella* was significantly different between treatments ($F_{3,44}$=4.42, p<0.01). The weight of pupae was affected by the PEEF treatment (Tukey´s HSD; p<0.05) but not by feeding on leaves treated with leaf extract (Tukey´s HSD; p=0.97) of *J. curcas*. In continuation with the previous results, the consumed leaf area during 4 days was significantly different between treatments ($F_{3,44}$= 10.77, p<0.001). The PEEF induced the highest protective effect for the leaf surface (Tukey´s HSD; p<0.001). In this treatment, the larvae fed on 3.96 ±0.10 cm^2 of the leaf surface. Although obviously less damaged compared to the control, the stem bark extract showed no significant inhibitory effect on the feeding performance of the larvae (Tukey´s HSD; p>0.05). In contrast to the laboratory experiment, the leaf extract did not show any protective effect of the leaf surface when applied in greenhouse conditions (Tukey´s HSD; p>0.05). In this case, the larvae damaged 4.65 ±0.14 cm^2 of the cabbage leaf surface during 4 days from the set-up of the bioassay.

4. Discussion

4.1. Contact toxicity of PEs and PEEF

The toxicity of oily compounds on *P. xylostella* has already been studied by Ratnadass *et al.* (2012) and Kumrungsee *et al.* (2014). However, the toxicity of the oil of *J. curcas* in particular was rarely investigated. According to our findings, the PEs caused a low mortality, when topically applied on the cuticle of the 2nd instar larvae of *P. xylostella*. However, the PEEF extract, mainly containing purified phorbol esters, caused a moderate mortality. Except the bioassays conducted by Wink *et al.* (1997), the toxicity of the PEs of *J. curcas* on the diamondback moth has not been so far reported. Wink *et al.* (1997) reported a mortality rate of 33% after 6 days when using the topical application of PEs. These results were supported by our experiment where the morality rate reached 26% after 6 days given a topical application of 2% of PEEF. Although no mortality was observed in our bioassay beyond 6 days after topical application, Wink *et al.* (1997) stated an increasing mortality to 60% after 13 days, which was not shown by our bioassay. The topical application of PEs and especially PEEF caused the larvae to become uncoordinated and convulsive. The dead larvae showed a necrotic cuticle with dark spots. These symptoms proved the cuticle irritating

effect of the tested compounds (Devappa *et al.,* 2010b) resulting in the damage of the integrity of cuticular waxes (Buteler *et al.,* 2011).

4.2. Ingestion toxicity of PEs and PEEF

The mortality caused by the ingestion of PEs and PEEF reached 28% and 38%, respectively. The PEs fraction was described as the most toxic compound contained in *J. curcas* (Goel *et al.,* 2007). The mortality of Lepidopteran larvae after administration of PEs based compound has been firstly reported by the study of Ratnadass *et al.* (2009). It attested an ingestion toxicity of PEs on all larval instars of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae), after treating the artificial diet with acetonic solution of PEs. Moreover, Devappa *et al.* (2012) reported a moderate ingestion toxicity of PEs and PEEF on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae), similar to our results on *P. xylostella*. This study showed a weak mortality rate that reached 40% after feeding on corn leaves treated with PEEF based extracts. The PEs are analogues of diacylglycerol, an activator of protein kinase C (PKC). PKCs act as regulators of many metabolic processes. As diacylglycerol has a short biological half-life in the cell, the activation of PKC is temporary. However, the activation of PKCs by PEs, is much extended, which subsequently disrupts a number of cell process and leads to the cell exhaustion (Kumar *et al.,* 2012). Devappa *et al.* (2011a) reported also that some Phorbol-type compounds (e.g. Jatropherol-1) increased the PKC activity of midgut silkworm *Bombyx mori* (Lepidoptera: Bombycidae), coupled with modifications in the function of several midgut enzymes and pathological alterations in midgut epithelial cells (Jing *et al.,* 2005).

4.3. Effect of the PEEF on the feeding behavior of the 1^{st,} 2nd and 3rd instar larvae

The data obtained from our bioassays clearly supported the fact that terpenoids such as phorbol esters are strong antifeedant/deterrent compounds against herbivores (Luo *et al.,* 2012). Several types of diterpenes (based on a 20-carbon skeleton) including phorbol esters were illustrated for affecting feeding behavior of insects and acting as gustatory deterrents (Devappa *et al.,* 2011b). The effect of PEEF on the 1st and 2nd instar of *P. xylostella* complied with the definition of deterrents advanced by Isman (1997). In our study, the PEEF acted as a behavior modifying substance that deterred feeding through a direct action on peripheral sensilla of larvae. This effect was perceived a short time after larvae came in contact with the applied compound (Isman, 2006).

The deterrence of larvae of *P. xylostella* after exposure to plant based products was shown in several studies. Shinoda *et al.* (2002) reported that the larvae of *P. xylostella* rejected the cabbage leaf discs treated with a triterpenoid saponin extracted from crucifer plant (*Barbarea vulgaris*). Also, Purwatiningsih *et al.* (2012) illustrated a larval deterrent effect after feeding on broccoli leaves treated with lemon-scented tea tree oil (*Leptospermum petersonii*). Moreover, Perera *et al.* (2000) and Liang *et al.* (2003) investigated the antifeedant properties of neem based products and found a high antifeedant/deterrent effect on larvae of *P. xylostella*. Concerning the PEEF, its deterrent effect was investigated by Devappa *et al.* (2012) on the fall armyworm *Spodoptera frigiperda* (Lepidoptera: Noctuidae). The results showed that the dietary intake by *S. frugiperda* was severely affected when fed on corn leaves treated with the PEEF.

In contrast with the 2 first instars, the PEEF did not show any deterrence on the 3rd instar and cabbage leaf discs were completely palatable by larvae. In fact, the old stages of *P. xylostella* are known to be resistant to insecticidal treatments (Gong *et al.,* 2013). The results of the study showed that the old larvae were obviously able to overcome the toxic effect of phorbol esters. The only known metabolic mechanism that could help larvae to feed on PEEF is the sequestration of toxic metabolites. Nishida *et al.* (2002) reported that some lepidopteran larvae sequester plant toxins during the larval stage and discard a large portion or all of the sequestrates when molting to a pupa or an adult.

Although its high toxicity on a wide range of insects, *J. curcas* is subject in the nature to herbivores infestation. Lama *et al.* (2015) drew-up the inventory of the pests of the physic nut tree and found 44% Hemiptera, 14% Coleoptera and 13% Lepidoptera, with mainly sucking and chewing insects. Moreover, Wink *et al.* (2000) reported the high ability of the aposematically coloured bug *Pachycoris klugii* (Heteroptera: Scutelleridae) to feed on *J. curcas*. The HPLC analysis of the hemolymph of this pest showed the presence of phorbol esters in the adults and old larval stages compared to eggs and neonates which suggested that old stages were more tolerant to PEs, as found in our study on *P. xylostella*.

The only study related to the sequestration of phorbol esters by Lepidoptera was reported by Marsh *et al.* (1984): Larvae of the spurge hawk moth (*Hyles euphorbiae*) feed on highly toxic Euphorbia plants and sequester cytotoxic ingenane diterpene esters. However, Hundsdoerfer (2005) showed that these larvae did not sequester the ingested phorbol esters since no accumulation of this toxin was found in the hemolymph and the teguments.

Contrary, the phorbol esters were mostly metabolized (about 70-90 %) and detoxified in the larval gut.

4.4. Anti-feedant effect of the PEEF, the stem bark extract and the leaf extract

The PEEF and the leaf extract of *J. curcas* showed an antifeedant effect on the 2nd instar larvae of *P. xylostella* in in vitro conditions. After 5 days of feeding on treated leaves, a clear inhibitory effect on the larval growth and the leaf consumption was observed. The screening for biocompounds from *J. curcas* by Blessing *et al.* (2011) showed the presence of high amounts of tannins, flavonoids, alkaloids and saponins; less present in the stem bark extract which could explained its inefficacy.

Tannins are typically defined as water-soluble phenolic compounds and protein-binding agents (Rossi *et al.,* 2004) in aqueous solutions (Salminen *et al.,* 2011). It was conventionally hypothesized that tannins decreased the digestion of proteins in insects and subsequently the growth performances (Barbehenn et *al.,* 2009a). The supposed digestion-reducing activity of tannins was ascribed to their capacity for precipitating the dietary proteins in the digestive tract (Barbehenn *et al.,* 2011) when high amounts of tannins were admixed to the insect food (Juntheikki, 2000). However, recent studies did not prove the effect of tannins on protein utilization when pure hydrolysable tannins and condensed tannins were administrated with tree leaves to the caterpillars of the gypsy moth *Lymantria dispar* (Lepidoptera: Erebidae) (Barbehenn *et al.,* 2009a).

Plants contain a variety of flavonoids that could influence insect behavior (Simmonds, 2003). Flavonoids are widely distributed in plants and known to be implied in resistance against pests attacks (Ohmura *et al.,* 2000). Flavonoids could modify insect feeding, fitness and development. Simmonds (2003) reported the antifeedant activity of some flavonoids and their related compounds on different Lepidopteran species including the African cotton leafworm *Spodoptera littoralis* (Noctuidae), the Black armyworm *Spodoptera exempta* (Noctuidae) and the tobacco Hornworm *Manduca sexta* (Sphingidae). Moreover, Ohmura *et al.* (2000) showed an antiffedant effect of flavonoid derived compound on the subterranean termite *Coptotermes formosanus* (isoptera: Rhinotermitidae). This ingestion of this compound might be also implied in the antifeedant effect against the larvae of *P. xylostella* in vitro.

The antifeedant effect of Saponins on Lepidoptera was reported by Dowd *et al.* (2011) on the corn earworm *Helicoverpa zea* (Lepidoptera: Noctuidae) and the fall armyworm

Spodoptera frugiperda (Lepidoptera: Noctuidae). The screening showed that the larval response to saponins was concentration dependent and each compound triggered a specific antifeedant effect on larvae. De Geyter *et al.* (2007) reported also that the larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) showed a strong inhibition of the larval growth after feeding on diet mixed with saponins. This phytocompound caused a low larval weight and an important delay in term of development time as seen on *P. xylostella*. Agrell *et al.* (2003) reported also that *S. littoralis* avoided feeding on alfalfa leaves damaged since 5 to 7 days due to the high amounts of saponins contained in leaves tissues.

Alkaloids have been shown to be strong antifeedants against Lepidopteran species (Isman, 1997) and could be involved in the low larval growth of *P. xylostella*. Kathuria *et al.* (2005) reported that alkaloids extracted from the leaves of Indian Ipecac plant *Tylophora indica* (Asclepiadaceae) and river red gum tree *Eucalyptus camaldulensis* (Myrtaceae) caused a dose-dependent feeding inhibition on the 5th instar larvae of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). Moreover, Shields *et al.* (2008) showed a high sensitivity of the gypsy moth larvae to 9 alkaloids. The two–choice feeding assay revealed an increase in feeding deterrence (decrease in consumption) with increasing concentration of alkaloid tested after exposing the 5th instar larvae to red oak treated leaf disks.

The aqueous leaf extract of *J. curcas* has not been so far reported for its antifeedant effect on insects, in particular on the diamondback moth. However, Bullangpoti *et al.* (2012) stated that the leaf extract of *J. gosspifolia* caused mortality when topically applied on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and decreased food intakes after being mixed with artificial diet. This study revealed also that the larval weight and the length of the biological cycle were highly impacted. Bullangpoti *et al.* (2012) showed also that the extract from the senescent leaves of *J. gosspifolia* inhibited detoxification enzymes including P450, general esterase and acetylcholinesterase activities, necessary to metabolize phytotoxic compounds.

In our bioassay, The PEEF of *J. curcas* acted as strong antifeedant compound against the larvae of *P. xylostella* in in vitro bioassays and under greenhouse conditions. This extract has been reported by Devappa *et al.* (2012) for its effect on the feeding behavior and growth of *S. frugiperda* after exposure to a range of PEEF concentrations. In contrast with PEEF which is an oily based compound, the water based leaf extract of *J. curcas* was rapidly

vaporized after application on the leaf surface. Moreover, the hydrophobicity of cabbage leaves might be another factor that explained the failure of the leaf extract in the greenhouse. Following application, the adaxial leaf surface was not perfectly coated with the extract. Subsequently, the clip-caged larvae detected the non-treated areas, make a hole on the leaves and move to the abaxial face where they feed superficially on the epidermis.

5. Conclusion

This study showed many aspects of the insecticidal properties of *J. curcas* on the diamondback moth *P. xylostella.* The investigation of the killing effect of the PEs and PEEF revealed a slight efficacy after topical and ingestion treatment. The PEs caused low mortality when topically applied on the cuticle or ingested by larvae of *P. xylostella* whereas the PEEF caused a moderate toxicity.

The PEEF and the leaf extract of *J. curcas* showed an antifeedant effect on the 2nd instar larvae of *P. xylostella* in in vitro conditions. The study supported the hypothesis that PEEF are strong deterrent compounds against herbivores. The supposed digestion-reducing activity of this compound was ascribed to the PEs diterpenoid fraction affecting the digestion of dietary nutrients when admixed to the insect food. PEEF was suggested to cause either a primary antifeedant effect (deterrence) by rejection of food, after short perception by taste gustatory receptors, or secondary antifeedant effect (post-ingestion).

Although having a wide spectrum of insecticidal aspects, the compounds of *J. curcas* were not frequently investigated. The killing and antifeedant properties of *J. curcas* could be valorized in the management of *P. xylostella* in Brassicaceae crops. The seed oil and the PEs extracted from *J. curcas* seeds could be promising alternative compounds to synthetic chemicals for the control of the diamondback moth. However, more toxicological studies and environmental assessments must be carried out to explore the side effects that these compounds could engender before their registration in crop protection.

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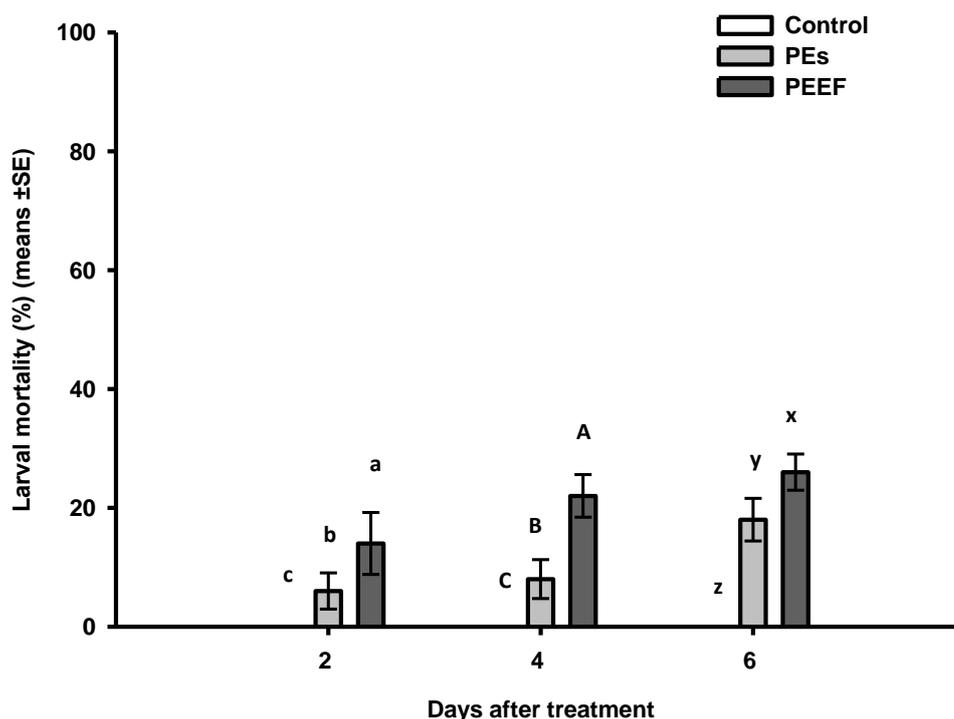
Figure 1: Topical toxicity of 2% of Phorbol Esters (PEs) and 2% of Phorbol Esters Enriched Fraction (PEEF) on 2nd instar larvae of *P. xylostella*. There was no mortality in the control group of larvae. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p <0.05).
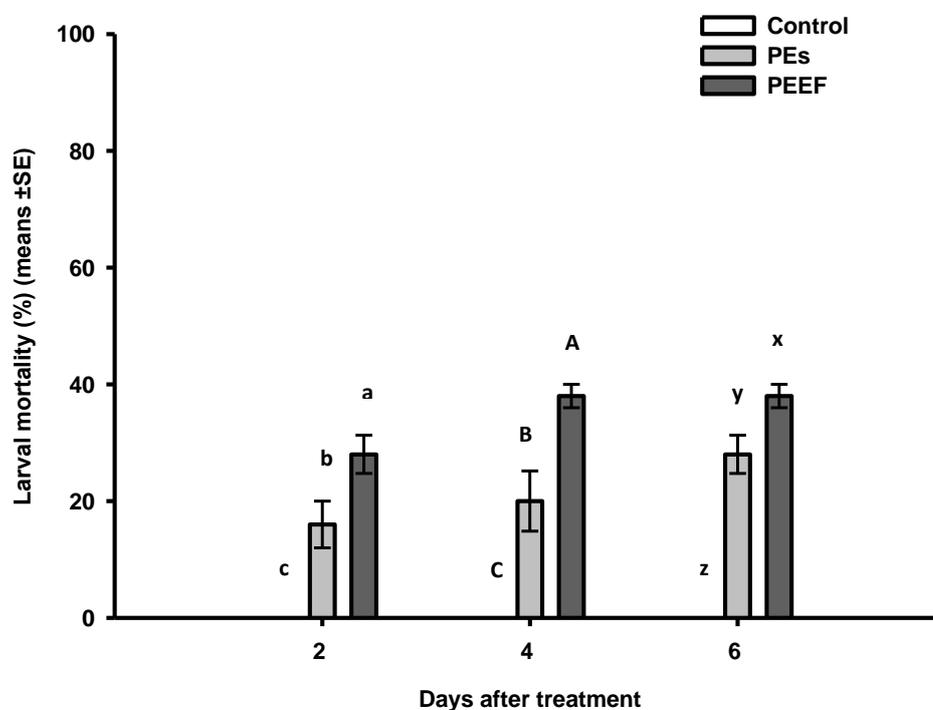
Figure 2: Ingestion toxicity of 2% Phorbol Esters (PEs) and 2% of Phorbol Esters Enriched Fraction (PEEF) on neonates larvae of *P. xylostella*. There was no mortality in the control group of larvae. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
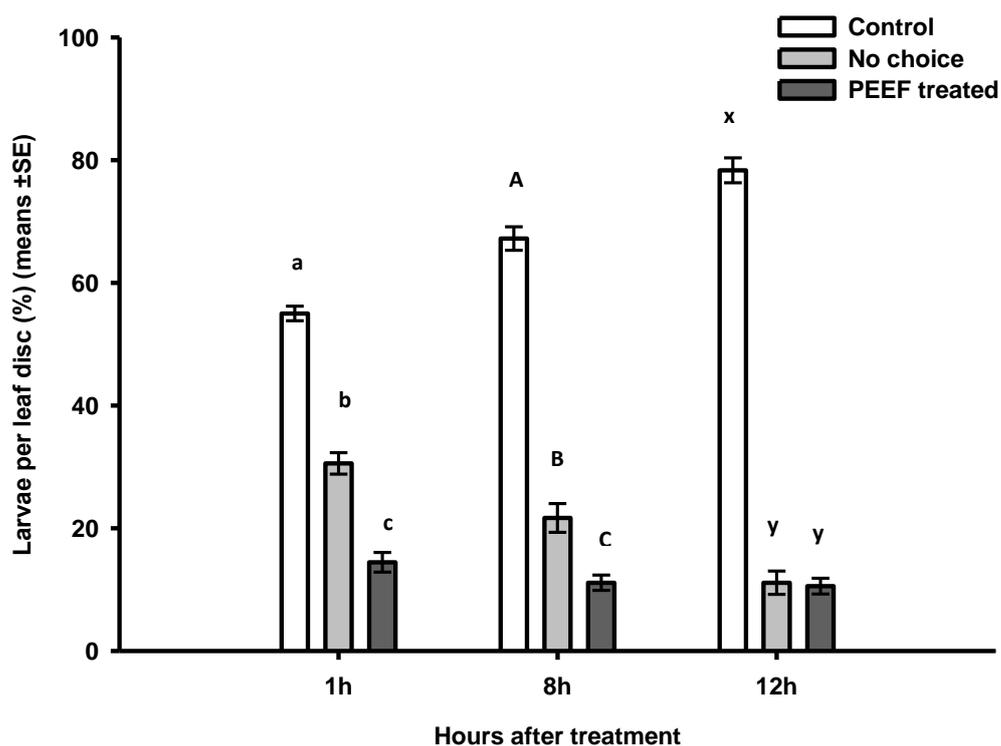
Figure 3a: Effect of 2% of PEEF on the feeding behavior of the 1st instar larvae of *P. xylostella* Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p <0.05).
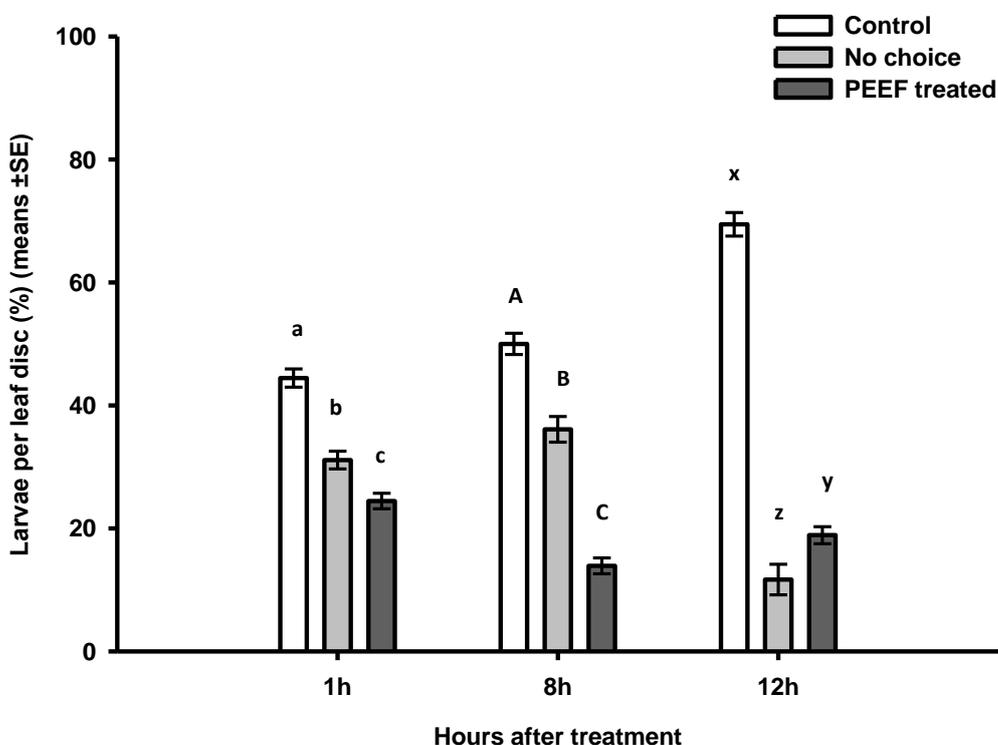
Figure 3b: Effect of 2% of PEEF on the feeding behavior of the 2nd instar larvae of *P. xylostella*. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p <0.05).
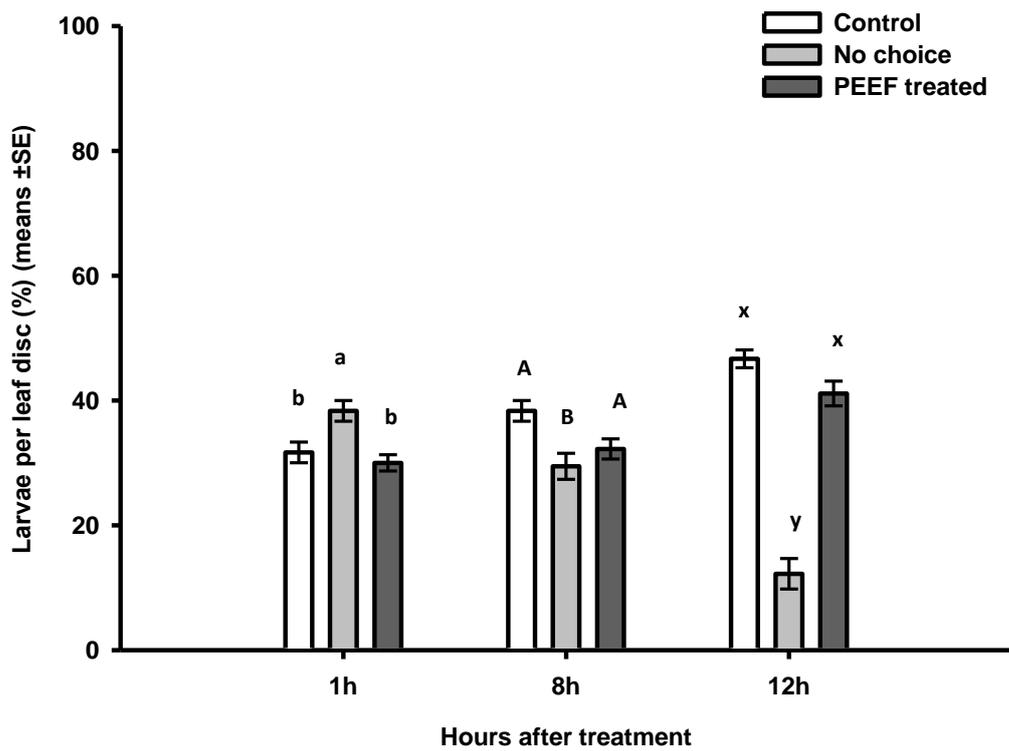
Figure 3c: Effect of 2% of PEEF on the feeding behavior of the 3rd instar larvae of *P. xylostella*. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, $p < 0.05$)

Chapter 4

Efficacy of some botanical extracts from the physic nut tree *Jatropha curcas* in the control of the cabbage root fly *Delia radicum* (CRF) (Diptera: Anthomyiidae).

Summary

Botanical insecticides have been long used as safe alternatives to synthetic chemicals for pest management. In this study, we assessed the insecticidal effect of the seed oil of *Jatropha curcas* (SOi) and the Phorbol Ester Enriched Fraction (PEEF) on the root feeding insect the Cabbage Root Fly *Delia radicum* (CRF) (Diptera: Anthomyiidae).

The ovicidal tests on *D. radicum* were carried out in Petri dishes. The method consisted of exposing 12 freshly laid eggs divided on 12 Petri dishes to 3 different concentrations of PEEF [0.5% (V/V), 1.25% (V/V) and 2% (V/V)] and SOi [0.5% (V/V), 1.25% (V/V) and 2% (V/V)] in two separated bioassays. Petri dish were sealed with parafilm and kept in dark phase in a climatic chamber at 22 ±1◦C and 60% RH. During incubation, the Petri dishes were daily observed under binocular and the embryos development was recorded. At the end of the bioassays, the number of eggs that failed to hatch after exposure to 1.25% and 2% of PEEF was significantly higher than the control (Tukey´s HSD; $p<0.001$). A concentration of 0.5% did not induce any ovicidal effect (Tukey´s HSD; $p=0.18$). The same inefficacy was observed after treatment with 0.5% of seed oil. However, a clear ovicidal effect was observed after exposure to 1.25% and 2% of SOi compared to the control (Tukey´s HSD; $p<0.001$).

The larvicidal effects of the PEEF and the SOi were tested on the neonate larvae of *D. radicum*. In this case, neonates were transferred in groups of 12 larvae to the surface of 12 turnip pieces as a food substrate already treated with Jatropha extracts. For that, one day before infestation with larvae, the pieces of turnip were dipped overnight in a Beaker filled with different solutions of PEEF [0.5% (V/V), 1.25% (V/V), 2% (V/V)] and SOi [0.5% (V/V), 1.25% (V/V), 2% (V/V)] and covered with an aluminum paper. After infestation, the pieces of turnip were placed in white boxes and covered with 2 cm height sterile sand. After 4th week, the sand of all treatments was sieved and the pupae were collected to deduce the pupation rate per treatment. The highest toxic effect was observed after feeding, during the larval stage, on turnip pieces treated with 2% of PEEF (Tukey´s HSD; $p<0.001$). The ingestion of 0.5% of PEEF did not affect the pupation success. In this treatment, no significant difference

to the control was detected (Tukey´s HSD; p=0.27). After feeding on the turnip treated with seed oil, the pupation success of larvae was not significantly reduced in the bioassays ($F_{3,44}$= 2.84, p>0.05). Feeding on 2% of the seed oil during the larval stage reduced the pupation success of larvae to 10.33 ±0.48 pupae (86.11%) pupae per box (Tukey´s HSD; p>0.05).

Under greenhouse conditions, cabbage plants were infested with 8 eggs of *D. radicum*. Eggs were carefully placed a few millimeters above the soil surface on the stem base of each cabbage plant and covered with some sand. After infestation, plants were drenched 2 times (1 day after infestation and 10 days after infestation) with 15 ml of PEEF applied around the stem base. There were 4 different treatments: the control plants neither infested nor treated, control plants infested but not treated, plants treated with 1.5% of PEEF and plants treated with 2% of PEEF. We observed that drenching the soil with a concentration of 2% of PEEF reduced significantly the number of pupae in comparison with the control (Tukey´s HSD; p<0.001). The roots of cabbage plants treated with 2% of PEEF were less damaged by the feeding activity of larvae and had significantly higher weight than the infested but not treated cabbage plants (Tukey´s HSD; p<0.001).

Keywords: *Jatropha curcas*, *Delia radicum*, Phorbol Esters Enriched Fraction, toxic seed oil, insecticide, root feeding insect.

1. Introduction

During the early years of use in crop protection, pesticides were considered to be generally safe with limited threats for health and environment. However, the intensive use of chemical insecticides and the rapid progress of agricultural techniques have led some major related issues to appear (Casida *et al.,* 1998). Pest resistance, ecological impacts and lasting health perils made it necessary to assess, review and frame the inappropriate use of these insecticides (Aktar *et al.,* 2009). These new concerns motivated the investigations into alternative pest control compounds to overcome or relatively reduce the repercussions generated by synthetic insecticides. Plant derived insecticide is regarded an alternative that could be investigated and applied in pest control programs (Isman, 2006).

Botanical insecticides have been long used as ecologically safe alternatives to synthetic chemicals for pest management. They are renewable, degradable in the environment and relatively safe to natural enemies, non-target organisms and human beings. Plants produce a range of chemical compounds to prevent attack by herbivores. Several extracts of plants have been evaluated for their activity against agriculturally important insects and being evaluated further for use in plant protection (Koul *et al.,* 2009). Such chemicals are secondary plant metabolites which include alkaloids, flavonoid and terpenoids.

The physic nut tree *J. curcas* possesses pesticidal properties. Several studies showed that extracts from *J. curcas* can be integrated in the management practices of agricultural pests (Acda *et al.,* 2009; Devappa *et al.,* 2012; Ratnadass *et al.,* 2012). The main insecticidal effect of this plant was ascribed to the phorbol esters, a diterpenoid fraction contained mainly in the seed oil (Devappa *et al.,* 2011). Extracts of the physic nut tree has been tested on a variety of insect species belonging to different orders: Hemiptera; Lepidoptera, Coleoptera, Diptera Orthoptera, Thysanoptera (Ratnadass *et al.,* 2012). Extracts that were studied and showed biological activity against insect pests of crops were mainly oil extracts, particularly phorbol esters tested on sap-sucking, leaf-chewing and root-feeding insects.

Root feeders are defined as insects feeding on belowground plant tissues during some part of their life cycle (usually as nymphs or larvae) including species feeding on root crowns (Blossey *et al.,* 2003). Root herbivores may disturb plant life cycle, affect the plant fitness and disrupt the food webs. Such a destructive root feeder is the cabbage root fly *Delia radicum* associated with Brassicaceae plants. The cabbage root fly *Delia radicum* (CRF)

is a pest of economic importance in cruciferous crops in North America and Europe (Bruck *et al.,* 2005). The economic losses due to root fly damage in these regions have been estimated to $100 million in some years (Shuhang *et al.,* 2016). Seedlings not protected by insecticides are often severely damaged by *Delia* pests. In some seasons, *D. radicum* infestations kill 90% of the plants in some untreated brassica crops in North America and mainland Europe (Finch, 1989). All pre-imaginal life stages including the 3 larval instars occur in the soil in the rhizosphere. After repairing the suitable host for oviposition, females lay eggs around the base of the cruciferous plants. After hatching, the larvae feed on the roots for about 3 to 4 weeks before pupating around the plant in the soil near to the surface. The number of generations of *D. radicum* depends upon climatic factors and there are usually three generations per year (Muška *et al.,* 2008). The adults of the overwintered last generation emerge in April-May, and the 2nd generation appears in late June to July (Finch, 1969). In Central Europe, the 3rd generation occurs before the end of the summer before overwintering as pupa in the soil.

The first generation of cabbage maggot is considered to be the most noxious to rapeseed, and most of the Brassicaecae vegetables (Muška *et al.,* 2008). In temperate zones root fly damage is important in spring and early summer (Griffiths *et al.,* 1986, Soroka *et al.,* 2011) when young plants and seedlings are infested by overwintered adult whereas in warmer climatic zones the root fly persists the whole year producing multiple overlapping generation throughout the year (Joseph *et al.,* 2014). The newly hatched larvae of *D. radicum* feed superficially on the root before burrowing into the root tissues with the increasing food requirements. This feeding behavior interrupts gradually the absorption and assimilation of nutrients and may lead to the reduction of yield (Jensen *et al.,* 2002). In spring, larval tunneling into the root system progressively weakens the plant and may cause the destruction of the seedlings (Fournet *et al.,* 2000). Larvae can also affect the quality of root crops such as turnip, making them non-commerciable. Moreover, the feeding injuries are potential pathways for fungal infection which may result in growth retardation or even plant mortality (Shuhang *et al.,* 2016).

Herbivores often cause extensive plant damages associated with secondary stress factors such as drought or grazing (Blossey *et al.,* 2003). However, the crucial lack of data about belowground pest makes them usually difficult to control (Hunter, 2001; Blossey *et al.,* 2003): they are hidden in the soil and therefore hard to detect and to reach (Kupferschmied

et al., 2013). The present research is an attempt to search for alternative compounds to control root-feeding pests. It consists on the investigation of the insecticidal effect of *J. curcas* extracts on the pre-imaginal stages of the cabbage root fly under laboratory and greenhouse conditions. The main tested compounds are the Phorbol Esters Enriched Fraction (PEEF) and the seed oil (SOi). We addressed the following questions:

- Does the PEEF and the SOi have a toxic effect on the eggs of *D. radicum* in laboratory conditions?

- Does the ingestion of PEEF or SOi induce a larvicidal effect on the neonates of *D. radicum*?

- Is PEEF less effective under greenhouse conditions compared to laboratory conditions?

2. Material and methods

2.1. Host plant

Cabbage plants *Brassica oleracea var. capitata L.* (cultivar: ``Furchenkohl´´; Norddeutsche Pflanzenzucht Hans-Georg Lembke KG) was used as host for the toxicity assays on the cabbage root fly *D. radicum*. The cabbage plants were grown in plastic trays from seeds in a greenhouse chamber (22 ±3°C temperature, 60% relative humidity and 16L: 8D photoperiod). After 10 days, seedlings were transplanted into 11 cm diameter pots filled with non-sterile soil (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-6.3, Fertilization of 200-300mg N) and sand mixture (3:1 volume). Plants were grown in the chamber, regularly irrigated and fertilized once a week (fertilizer: Hakaphos 2g/L (15% N, 11% P2O5, 15% K2O, 1% Mg, 0.1% Fe, 0.1% Mn, 0.04% Cu, 0.025 %B and 0.005 % Mo)).

2.2. Insect culture

The cabbage root fly was obtained from a laboratory stock culture maintained in a climate rearing room (21 ±1°C temperature, 60 ±5% relative humidity and LD 16h: 8 h photoperiod). This laboratory rearing was established from flies originally provided by the JKI (Julius-Kühn-Institut, Braunschweig, Germany) in November 2003. Adult flies were detained in cubic screened cages (30 cm × 30 cm × 30 cm) and fed with artificial diet following the method of Finch *et al.* (1969) with some modifications: A cube of Kohlrabi (100 g) (*Brassica oleracea var. gongylodes*) was placed on a layer of washed quartz sand in a Petri dish to attract the females and stimulate the oviposition in the nearing sand. The sand was mixed

with water and the eggs were collected by floating. The water containing the floating eggs was filtrated in a funnel through a filter paper then transferred to a fresh kohlrabi piece with a shallow cut-surface. The kohlrabi was covered with sand and placed for 3 weeks in white boxes (18cm length × 13cm width ×5.5cm height) until the pupation of larvae then pupae were collected by floating out of the sand. Two types of food were provided to the adults: dry powder and sticky honey. The powdery food was made of dextrose, organic skim milk powder, soy flour and brewer's yeast (10g:10g:1g:1g); the sticky honey was made of commercially available honey, soy flour and brewer's yeast (5g:5g:1g). Adults were provided with water in a moistened filter paper.

2.3. Jatropha bioinsecticides

In this study, two different extracts of *J. curcas* were screened for their insecticidal effect: the seed oil (SOi) and the Phorbol Ester Enriched Fraction (PEEF). The oils were provided by the company of JatroSolutions GmbH (Stuttgart, Germany) where they have been extracted and purified following the method of Devappa *et al.* (2010a) then stocked in a cold room to be used in bioassays.

2.4. Experimental bioassays

2.4.1. Ovicidal effect of the PEEF and the seed oil (SOi)

This bioassay was conducted in in vitro conditions. Freshly laid eggs of *D. radicum* were collected by the floating method from a laboratory culture. The eggs were observed under binocular (Leica, Wild, M3Z, Wetzlar, Germany) to sort out the healthy eggs for the experiment. The bioassay consisted of 4 different treatments: The control group exposed to bidest water, the eggs exposed to 0.5% (V/V), 1.25% (V/V) and 2% (V/V) of the PEEF. The toxicity test was done in Petri dishes (5.5 cm diameter x 1.5 cm height) and consisted of exposing eggs of *D. radicum* to different compounds of *Jatropha* on a filter paper (50 mm diameter, MN 615) (Macherey-Nagel GmbH, Düren, Germany) imbibed with one of the bioinsecticides (the seed oil and the PEEF). Eggs were carefully collected with camel brush, placed in each Petri dish then sealed with Parafilm M® (Bemis Company, Inc., Neenah, USA) and kept in dark phase in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1∘C and 60% RH. During incubation, the Petri dishes were daily observed under binocular and the development of embryos was recorded. The number of eggs that failed to hatch was recorded to assess the ovicidal effect of the compounds. The

same methodology was followed and the same concentrations as the PEEF were tested to assess the toxicity of the seed oil on the eggs of *D. radicum*. Every treatment had 12 repetitions (Petri dishes) with 12 eggs and the experiment was done once.

2.4.2. Larvicidal effect of the PEEF and the seed oil (SOi)

Fresh laid eggs of *D. radicum* were placed in groups of 20 eggs in Petri dishes (5.5 cm x 1.5 cm) lined with a moistened filter paper (50 mm diameter, MN 615) for hatching. The Petri dishes were sealed with Parafilm M® and incubated in dark phase in a climatic chamber at 22 ±1∘C and 60% RH. After hatching, filter papers were moistened with 500 µl of sterile bidest water, the larvae were shortly flooded and gently collected with a pipette (Eppendorf AG, Hamburg, Germany). The neonates were transferred in groups of 12 larvae to the surface of turnip pieces already treated with *Jatropha* extracts. One day before inoculation with larvae, the pieces of turnip (*Brassica napus subsp. Rapifera*) were cut in cubes of 50 g weight and dipped overnight in a beaker (200 ml) filled with different solutions of PEEF [(0.5% (V/V), 1.25%(V/V) and 2%(V/V)] and SOi [(0.5%(V/V), 1.25% (V/V) and 2%(V/V)] and covered with an aluminum paper. Some superficial crossing lines of 1 cm depth were made on the surface of turnip to facilitate the first food intakes of the neonate larvae of *D. radicum*. In the following day, the pieces were removed with forceps from the bioinsecticide solutions and left to dry for 30 mn on sterile paper towels (TORK, Mannheim, Germany) in laboratory conditions before being infested with larvae.

After infestation, the pieces of turnip were placed in white boxes (18cm length × 13cm width ×5.5cm height) on a 3 cm height layer of sand and covered again with 2 cm height sterile sand autoclaved at 120∘C. The boxes were placed in controlled room conditions (22 ±1 ∘C temperature, 16:8 h (L: D) and 60% RH) and sprayed twice a week with sterile water to avoid turnip dryness. During the 4th week after the set-up of the bioassay, the sand surface of the control treatment was stirred to check the presence of pupae which indicated the end of the experiment. The sand of all treatments was sieved and the pupae were collected and counted to deduce the pupation rate per treatment. Pupae were then individually incubated in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1∘C and 60% RH to observe their emergence success. There were 4 different treatments per bioinsecticide; each treatment was replicated 12 times in a completely randomized design.

2.4.3. Insecticidal effect of PEEF under greenhouse conditions

The objective of this experiment was to assess the efficacy of *Jatropha* compounds in semi-real agricultural conditions. For that, 4 weeks old cabbage plants grown in 11 cm pots were used in this bioassay. Plants were grown from seeds in soil (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-6.3, Fertilization of 200-300mg N) and sand mixture (3:1 volume) as a growth substrate. Freshly laid eggs of *D. radicum* were collected from a stock colony and kept on a moistened filter paper at 22∘C. After 24 hours, healthy eggs were collected under binocular and used in this bioassay whereas the others were discarded.

In order to not expose the plants to a high larval feeding pressure, cabbage plants were infested with only 8 eggs. Eggs were carefully placed with a camel fine brush few millimeters above the soil surface on the stem base and covered with some sand. After infestation, plants were drenched 2 times (1 day after infestation and 10 days after infestation) with 15 ml of PEEF applied around the stem base. There were 4 different treatments: the control plants neither infested nor treated, control plants infested but not treated, plants treated with 1.5% (V/V) of PEEF and plants treated with 2% (V/V) of PEEF. TWEEN® 80 was used as emulsifier. After 4 weeks of the experiment set-up, plants were harvested and the pupae were collected. The roots were washed under running water, the sand was twice filtered (sieve №4 (4.75 mm opening) and sieve №10 (2 mm opening)) and pupae were collected with forceps in labelled Petri dishes. Pupae were left to dry in Petri dishes (90 mm x 14.5 mm) (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany) lined with a filter paper, counted then weighed and measured. Plants were oven dried at 60 ∘C (Model 30-106. Memmert GmbH + Co. KG, Schwabach, Germany) for one week to determine their biomass and assess damages. The experiment was conducted under greenhouse conditions (22 ±3°C temperature, 60% relative humidity and 16L: 8D photoperiod) with 10 plants per treatment in a completely randomized design.

The pupae of the previous bioassay were incubated in white plastic box (18cm length ×13cm width ×5.5cm height) in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) at 22 ±1∘C and 60% RH. After recording the emergence rate, adults from the 3 treatments were released in 3 egg-laying cages (30 cm x 30 cm x 30 cm) and provided with two types of diet: dry powder and sticky honey. The powdery food was made of dextrose, organic skim milk powder, soy flour and brewer's yeast (10g:10g:1g:1g);

the sticky honey was made of commercially available honey, soy flour and brewer's yeast (5g:5g:1g). Adults were provided with water in a reversed glass put upside down on a filter paper on the lid of a sterile Petri dish (90 mm x 14.5 mm). As oviposition support, a cube of kohlrabi (100 g) (*Brassica oleracea var. gongylodes*) was placed on a layer of washed quartz sand in a Petri dish to attract the females and stimulate the oviposition in the nearing sand. After 5 days, the Petri dishes were removed, the sand was mixed with water and the eggs were collected by floating followed by filtration. The eggs were counted under a binocular (Leica, Wild, M3Z, Wetzlar, Germany) to determine the fecundity of females. This bioassay was conducted in a controlled conditions room at 22 ±1 ∘C, 16:8 h (L: D) and 60% RH. There were 3 cages with 12 couples of *D. radicum*.

2.5. Data analysis

Statistical processing of results was carried out by standard methods using the statistical software R (v 3.0.2) (R Development Core Team, 2008). Prior to analysis, all data were tested for homogeneity of variance and normality. To detect the difference between treatments, we performed One-way analysis of variance (ANOVA). If significant differences between groups were detected, means were compared by performing Tukey's test at α = 0.05. The mortality of insects after treatment with the extracts of *J. curcas* (seed oil, PEEF or PEs) was analyzed using generalized linear models (GLM) taking account of the binomial distribution of the data (alive or dead). To correct the anomalies in data variation (overdispersion / underdispersion), we proceeded to the quasi-binomial distribution error in data analysis. Thereafter, Tukey's test at α = 0.05 was employed to detect differences between means.

3. Results

3.1. Ovicidal effect of the PEEF and the seed oil (SOi)

The exposure of the eggs of *D. radicum* to PEEF showed a significant difference between concentrations in term of hatching rate ($F_{3,44}$=31.47, p<0.001; Figure 1a). Many eggs significantly failed to hatch after being treated with 2% of PEEF in in vitro conditions (Tukey's HSD; p<0.001). In the control treatment and after exposure to 2% of PEEF hatched respectively 11.16 ±0.27 eggs (93.05%) and 6.33 ±0.33 eggs (52.77%) after incubation. The treatment with 1.25% of PEEF reduced the eggs hatchability to 8.33 ±0.39 eggs (69.44%) which was significantly different from the control (Tukey's HSD; p<0.001). A Concentration

as low as 0.5% of PEEF did not induce any potent ovicidal activity (Tukey´s HSD; p=0.18). We recorded 10.25 (85.41%) hatching eggs. A correlation was evident between the eggs hatchability and the concentration (R = 0.81, *df* = 46, p <0.001, Pearson correlation).

The seed oil of *J. curcas* caused a significantly different mortality between treatments ($F_{3,44}$=22.01, p<0.001; Figure 1b). At a concentration of 2%, 5.83 ±0.48 eggs (48.61%) hatched which is significantly lower than the control where it reached 10.5 ±0.28 eggs (87.5%) (Tukey´s HSD; p<0.001). Egg mortality was concentration dependent. The toxic effect became lower after exposure to a concentration of 1.25%. In this case, we observed a moderate ovicidal effect of the seed oil but it was still effective compared to the control (Tukey´s HSD; p<0.001). The treatment of eggs with 1.25% of the seed oil of *J. curcas* reduced their hatchability to 7.58 ±0.49 eggs (63.19%) among the 12 eggs initially exposed to the bioinsecticide. In comparison with the control, no significant effect was observed at 0.5% of seed oil on the eggs. We recorded a hatching rate of 9.75 ±0.31 eggs (81.25%). Also, no correlation was detected between the rate of hatching failure and the concentration of the seed oil (R = 0.02, *df* = 46, p =0.87, Pearson correlation).

3.2. Larvicidal effect of the PEEF and the seed oil (SOi)

The pupation rate induced by the ingestion of PEEF differed significantly between treatments ($F_{3,44}$= 36.05, p<0.001; Figure 2a). The highest toxic effect was observed after feeding, during the larval stage, on turnip pieces treated with 2% of PEEF (Tukey´s HSD; p<0.001). This concentration reduced the pupation to 5.66 ±0.37 pupae (47.22%) which was much lower than the control treatment recording 11.25 ±0.35 (93.75%) of pupated larvae. Only 7.83 ±0.45 larvae (65.27%) fed on 1.25% of PEEF reached the pupal stage. The ingestion of 0.5% of PEEF did not affect the larval survival and the pupation success. In this treatment, no significant difference to the control was detected (Tukey´s HSD; p=0.27).

Pupation rates were higher after treatment with seed oil in comparison with PEEF. After feeding on the turnip treated with seed oil, the pupation success of larvae was not significantly reduced in the bioassays ($F_{3,44}$= 2.84, p=0.058; Figure 2b). After ingestion of 2% of seed oil, the statistical analysis showed a non-significant difference with the control. Feeding on 2% of the seed oil during the larval stage reduced the pupation success of larvae to 10.33 ±0.41 (86.11%) pupae per box (Tukey´s HSD; p=0.065). There was also no significant difference in the number of pupae when larvae develop on turnip treated with 1.25% of seed oil. We collected 10.5 ±0.28 pupae out of the 12 larvae initially released on the pieces

of turnip (Tukey´s HSD; p=0.11). Moreover, larvae fed on 0.5% of seed oil were not affected in comparison with the control and 11.08 (92.36%) pupae were collected from the sand.

3.3. Insecticidal effect of PEEF on *D. radicum* under greenhouse conditions

Pupation rates of *D. radicum* differed significantly between treatments ($F_{2,27}$= 161.5, p<0.001). The PEEF showed a high inhibitory effect of the pupation of the cabbage root fly under greenhouse conditions (Figure 3a). Drenching the soil with a concentration of 2% of PEEF reduced significantly the number of pupae in comparison with the control (Tukey´s HSD; p<0.001). We collected in the control and the PEEF treatment respectively 7.6 ±0.16 pupae (95%) and 2.9 ±0.23 pupae (36.25%).

When applied on the soil surface on the base of cabbage plants, the concentrations of 1.5% affected the egg hatching and/or larval development and led to a significant decrease of the pupation rate (Tukey´s HSD; p<0.001). At this concentration, only 4.7 ±0.15 pupae (58.75%) out of the 8 eggs, initially used for plant infestation, pupated. The statistical analysis showed also no significant difference in the pupal weight of males ($F_{2,67}$= 0.70, p=0.49) and females pupae ($F_{2,67}$= 1.53, p=0.22) between treatments. After incubation in the climatic chamber, only one pupa collected from cabbage plants treated with 2% of PEEF did not emerge. The fecundity of females was not affected by this *Jatropha* compound. The number of eggs laid in every egg laying cage was similar to the control (Tukey´s HSD; p>0.05), collected by floating from the sand used as oviposition substrate.

The root dry weight of the cabbage plants was significantly different between treatments ($F_{3,36}$= 93.88, p<0.001; Figure 3b). The roots of infested non-treated plants were heavily damaged by the feeding activity of the larvae and had significantly lower weight than the cabbage plants treated with 2% of PEEF (Tukey´s HSD; p<0.001). The soil application of a concentration of 1.5% of PEEF protected significantly roots from the larval damages in comparison with the control (Tukey´s HSD; p<0.01) but showed lower efficacy than drenching the soil with 2% of PEEF (Tukey´s HSD; p<0.01). No significant difference was observed between root dry weight of the cabbage plants treated with 2% of PEEF and non-infested cabbage plants (Tukey´s HSD; p=0.60).

4. Discussion

4.1. Ovicidal effect of the PEEF and the seed oil (SOi)

The PEEF and the seed oil of *J. curcas* showed toxicity on the eggs of *D. radicum* in in vitro conditions. The eggs treated with high concentrations of these compounds developed normally until the 3rd day before they started to turn brown. The eggs that showed a change in color did not hatch. Plant oils have been reported for their ovicidal effect on different insect species. Tripathi *et al.* (2003) obtained 99.5% reduction in egg hatching after applying the essential oil of the bael tree *Aegle marmelos* on the eggs of *Spilosoma obliqua* (Lepidoptera: Erebidae). Malarvannan *et al.* (2009) showed that the oil of *Clausena dentata* had a high ovicidal effect when topically applied on the eggs of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae)*.*

Some plant oils have been reported for their ovicidal effect on Dipteran species such as *D. radicum*. Prowse *et al.* (2006) reported that the garlic juice concentrate caused significant mortality in eggs and adults of *Delia radicum* and *Musca domestica*. Kumar *et al.* (2013) revealed also a high potential of the essential oil of *Cymbopogon citratus* (Poales: Poaceae) in controlling house flies. Furthermore, Govindarajan *et al.* (2011) showed a strong ovicidal effect of *Caesalpinia pulcherrima* (Fabales: Fabaceae) extract at a concentration of 5.0 mg/cm^2 on the eggs of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae).

The ovicidal effect of *J. curcas* has been reported by few studies. Agboka *et al.* (2009) showed a high toxicity of the seed oil on the eggs of the maize cob borer *Mussidia nigrivenella* (Lepidoptera: Pyralidae). As shown by our study, their findings showed also concentration dependent toxicity with L$_{50}$ of 1.3% of the seed oil of *J. curcas*. Moreover, Khani *et al.* (2012) revealed significant reductions in egg hatchability after treatment of the eggs of the rice meal moth *Corcyra cephalonica* (Lepidoptera: Pyralidae) with petroleum ether extract of *J. curcas*. In this case, the reduction of hatchability reached 58% after mixing rice with 2 µL/mL petroleum ether extract of seed oil. Buteler *et al.* (2011) suggested that oils induced the eggs mortality by suffocation. Essential oils create a thin layer on the surface of eggs and stop the gas exchanges. They might also cause hardness in eggs which make it difficult for larvae to hatch.

4.2. Larvicidal effect of the PEEF and the seed oil (SOi)

The PEEF induced a reduction of the pupation rate of the larvae of *D. radicum* after feeding on a concentration of 2% of PEEF. This effect was not observed after feeding on the pieces of turnip treated with the seed oil of *J. curcas*. It was suggested that the toxic effect of PEEF was generated by the combined toxicity induced by the pure PEs fraction and the fatty acids complex contained in the extract. Devappa *et al.* (2013) reported that among its richness with highly purified PEs, the PEEF contained also an amount of fatty acids such as palmitic, palmitoleic, stearic, oleic and alpha-linoleic acid, which was not the case of the seed oil containing low amounts of PEs.

The ingestion toxicity of PEs has been reported by few studies. The larval toxicity after ingestion of PEs rich compounds has been first mentioned by Ratnadass *et al.* (2009) on the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). They reported a significant toxicity of PEs by feeding bioassays on all larval instars, after treating the artificial diet with acetonic solution of this compound. In addition, Devappa *et al.* (2012) observed an ingestion toxicity of PEEF on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) after feeding on corn leaves dipped in a solution containing 0.25 mg/ml of PEs. In our study, the moderate toxicity of the PEs has been already shown on the diamondback moth *P. xylostella* after feeding on cabbage leaf discs treated with PEEF.

Fatty acids have been also reported for their insecticidal effect. Ramos-López *et al.* (2012) showed that linolenic and linoleic acids purified from the castor-oil plant (*Ricinus communis*), and also highly present in the oil of *J. curcas*, exhibited insectistatic and insecticidal activities against *Spodoptera frugiperda*. Moreover, Mat Sarip *et al.* (2016) showed that in addition to its contact toxicity on *Aphis gossypii*, the ingestion of lauric acid present also in the seed oil of *J. curcas*, could also affect insect´s growth and induce mortality. Also, Pérez-Gutiérrez *et al.* (2011) found that oleic, palmitic and stearic acids extracted from Papaya (*Carica papaya*) were toxic to the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) when admixed to the artificial diet of this pest and caused the pest starvation leading to death.

4.3. Insecticidal effect of PEEF under greenhouse conditions on *D. radicum*

PEEF was efficient under greenhouse conditions at 2% concentration. As it was observed in in vitro conditions, the number of larvae that reached the pupal stage was significantly reduced after treating the soil with this compound. In relation with the previous

results, this decrease of survival could occur during the larval stage when feeding on PEEF treated roots or earlier during the egg development phase. The PEEF has been proved by Devappa *et al.* (2012) to cause an antifeedant effect on the third instar larvae of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). It was also shown in our study that this compound induced an antifeedant / deterrent effect on the diamondback moth *P. xylostella* (Lepidoptera: Plutellidae) and led either to mortality by starvation or formation of small pupae. However, in this bioassay the pupal weight of *D. radicum* was not affected after feeding during the larval stage on PEEF. This suggested that PEEF did not have a long time efficacy when mixed to the soil and larvae could feed normally on roots.

Devappa *et al.* (2010b) and Roach *et al.* (2012) studied the biodegradation of PEs with different approaches and demonstrated that the shelf-time of this compound was widely dependent on the soil characteristics and the bioassay conditions (temperature, moisture or light). Devappa *et al.* (2010b) showed also that the seed cake of *J. curcas* was rapidly degraded in non-autoclaved than in autoclaved soil, which suggested that the soil fauna played a major role in the degradation process. In addition, Najjar *et al.* (2014) showed that PEs of *J. curcas* were completely degraded by *Trichoderma* spp., a genus of fungi communally present in agricultural soils, *Paecilomyces* sp. and *Cladosporium* sp.. and concluded that these fungi were potential microbes for the detoxification of PEs.

Devappa *et al.* (2010b) demonstrated by HPLC analysis that the PEs in soil were completely degraded after 9 days at a temperature of 32ºC and 12 days at 23 ºC. In our case, giving that the PEEF was applied twice on the 1st and the 10th day of the bioassay, this was not sufficient to cover the whole biological cycle of *D. radicum* lasting about 30 days. Because of its short shelf-time, the application of PEEF under greenhouse conditions would cover only 20 days of the life cycle of *D. radicum* before being completely degraded. It was hypothesized that at the end of the bioassay, PEEF lost its antifeedant property and allowed the aged surviving larvae to feed on roots and reach a normal pupal weight. Subsequently, the plant growth was not affected because the feeding activity occurred tardily when the root system was well developed and tolerate the damages caused by the larvae of *D. radicum*. Also, it was supposed that the mortality of immature stages occurred at the egg and early larval stages when the PEEF was still effective.

5. Conclusion

This study showed the potential effect of *J. curcas* as a plant derived insecticide on root-feeding insects. The PEEF and the seed oil of *J. curcas* showed toxicity on the eggs of *D. radicum* in in vitro conditions. Essential oils create a thin layer on the surface of eggs surface and stops the gas exchanges. They might also cause hardness in eggs which make it difficult for larvae to hatch. The PEEF induced also a reduction of the pupation rate of the larvae of *D. radicum* after feeding on a concentration of 2% of PEEF. These extracts from *J. curcas* induced a killing effect depending on the concentration of PEs. The corn root worm showed more tolerance to the compounds of *J. curcas*. These results showed that the extracts of *J. curcas* in particular the PEEF could be used at a certain extend as an alternative to chemical insecticides. The limited longevity of these extracts might be enhanced by association with other plant protection agents that could have an additive or synergetic activity when mixed with these compounds.

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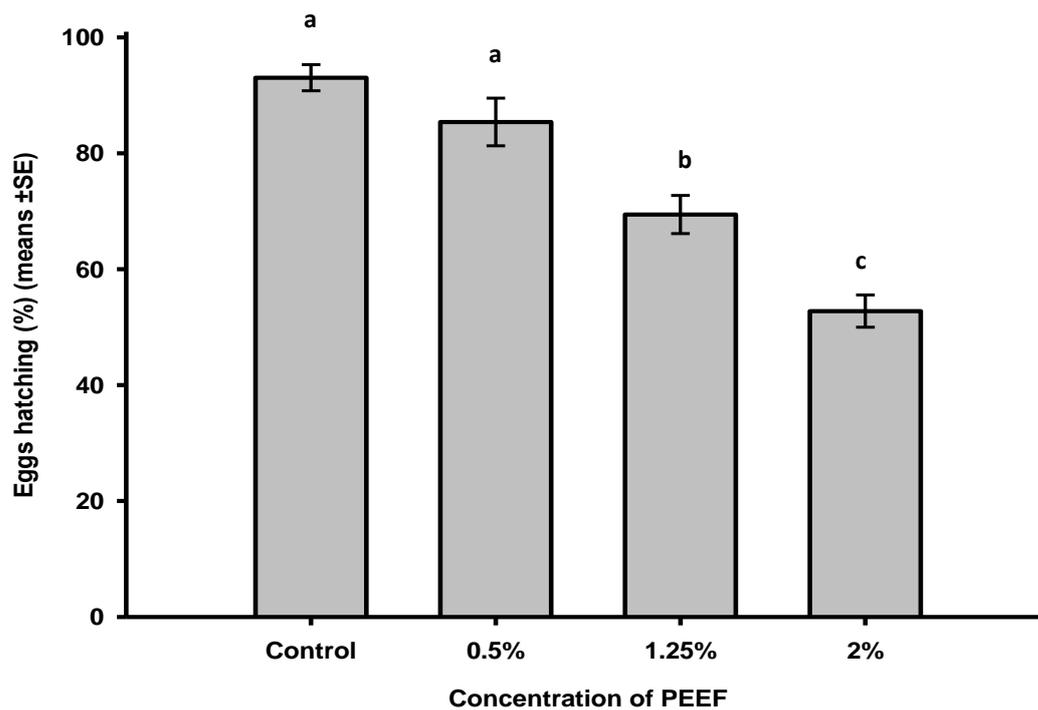
Figure 1a: Ovicidal effect of the Phorbol Esters Enriched Fraction (PEEF) on the eggs of *D. radicum*. Bars denoted with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
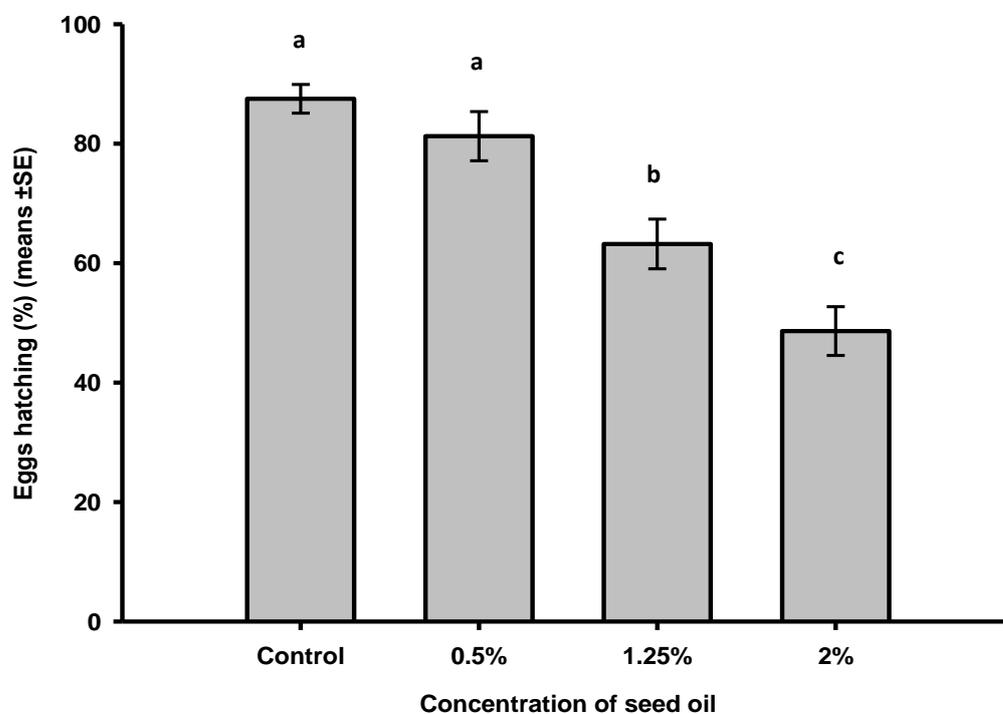
Figure 1b: Ovicidal effect of the seed oil of *J. curcas* on the eggs of *D. radicum*. Bars denoted with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
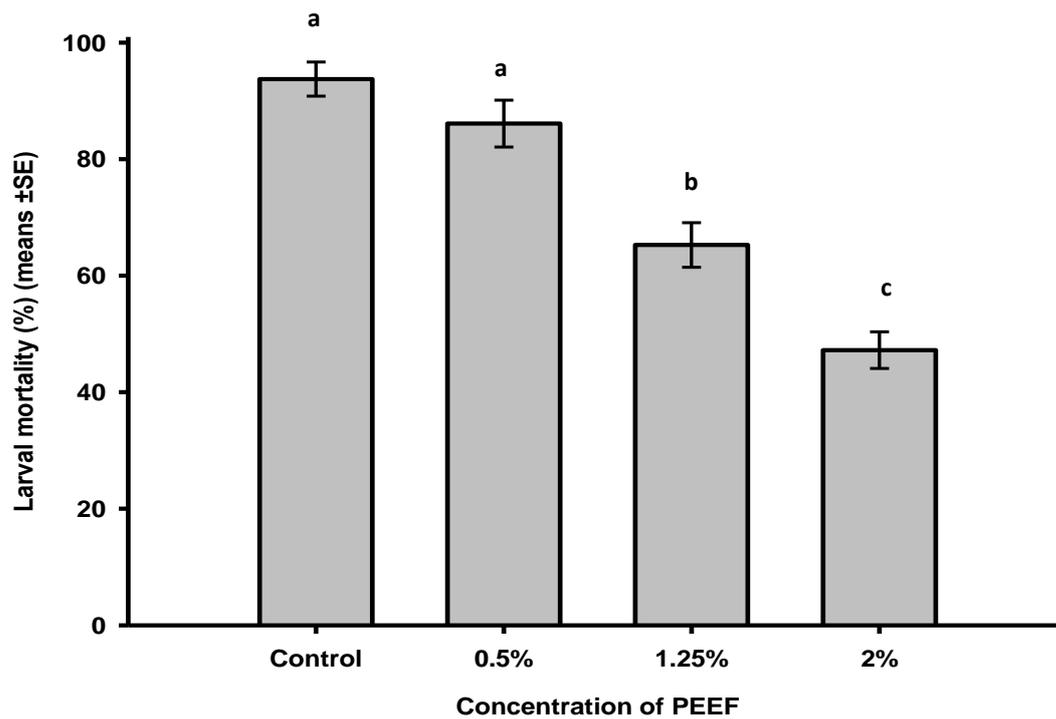
Figure 2a: Larvicidal effect of the Phorbol Esters Enriched Fraction (PEEF) on the larvae of *D. radicum*. Bars denoted with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
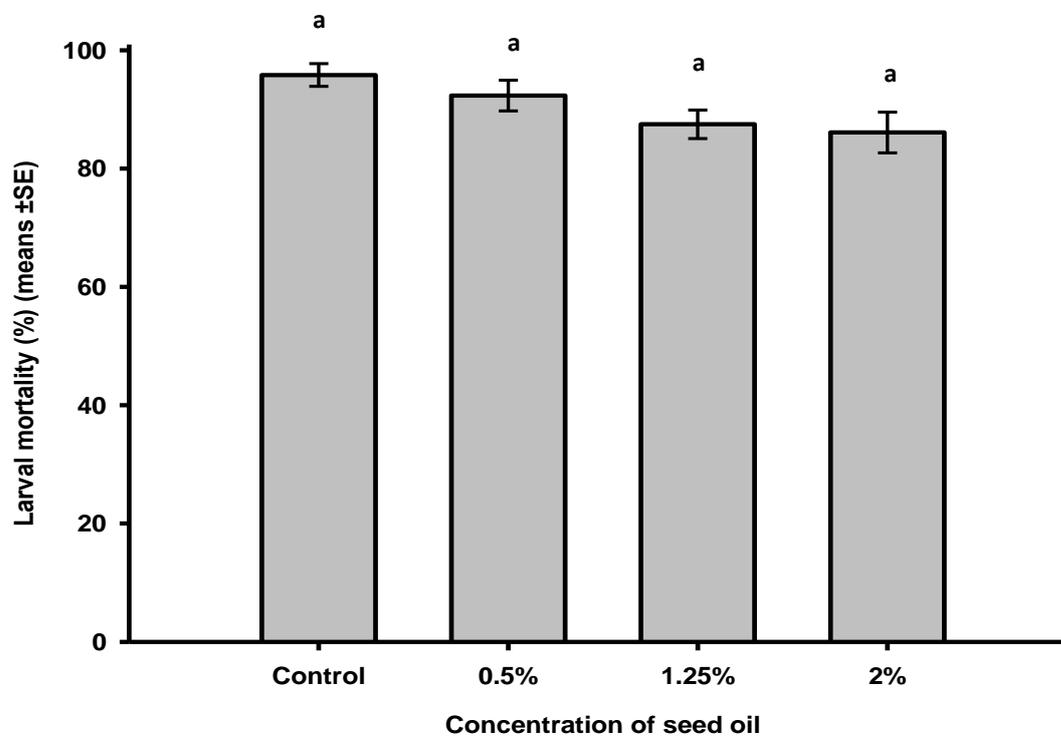
Figure 2b: Larvicidal effect of the seed oil of *J. curcas* on the larvae of *D. radicum*. Bars denoted with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05)
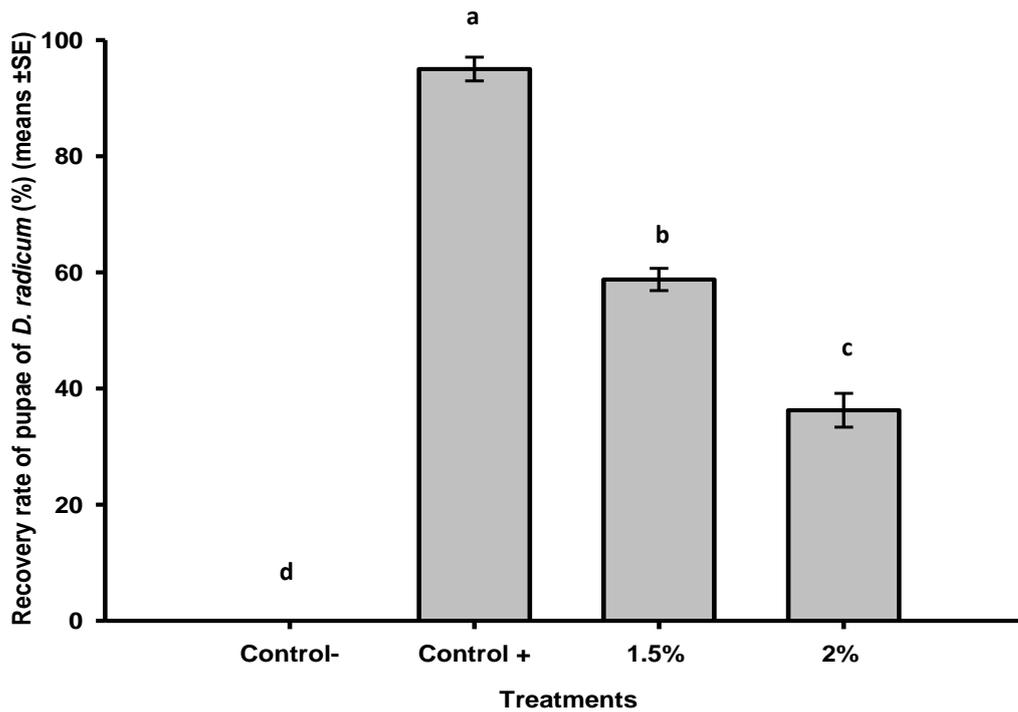
Figure 3a: Recovery rate of the pupae of *D. radicum* on cabbage plants after treatment with PEEF under greenhouse conditions. Bars denoted with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
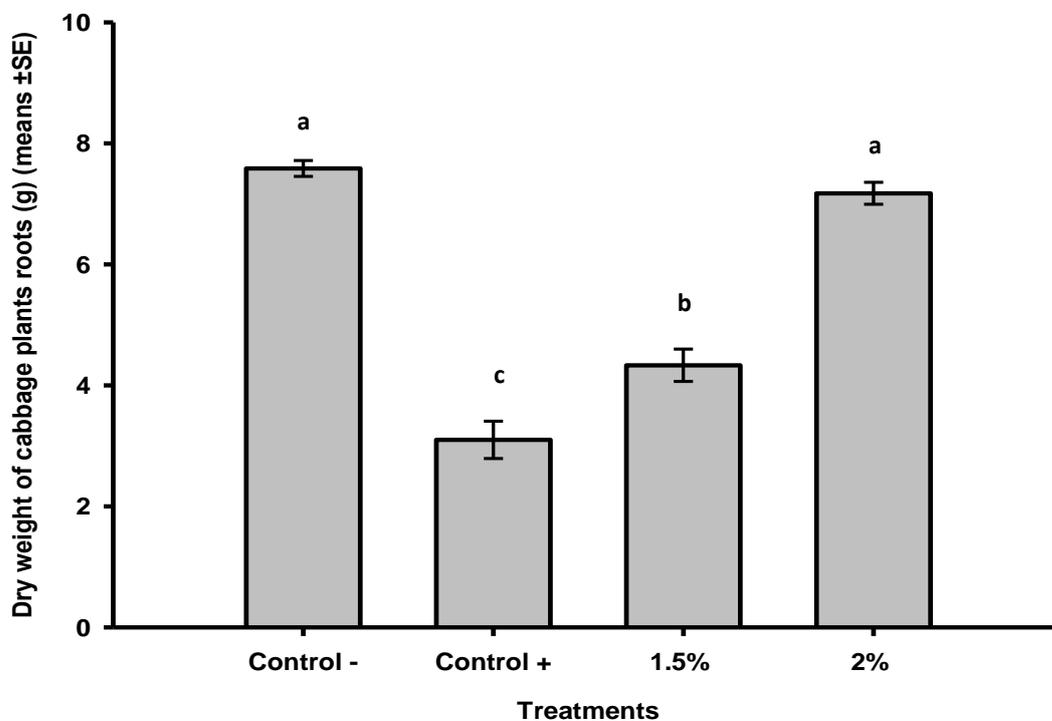
Figure 3b: Dry weight of the roots of cabbage plants after treatment with PEEF under greenhouse conditions. Bars denoted with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05)

Chapter 5

## Potential use of some biocompounds extracted from the physic nut tree *Jatropha curcas* in the control of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae)

Summary

The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is an economically important pest in field and greenhouse crops. Its major pest status derived from its polyphagy, high fecundity and mobility. Various botanical extracts have been reported to have a wide toxicity spectrum on *H. armigera*. However, only some botanical insecticides are used in crop protection, such as the physic nut tree *Jatropha curcas* well known for its toxicity but underutilized in pest management programs. The aim of the present study was to assess the insecticidal effect of this plant on *H. armigera* and show the different pesticidal aspects of the Phorbol Esters Enriched Fraction (PEEF) and the seed oil (SOi).

The contact toxicity of PEEF was tested on the 2nd and 4th instar larvae of *H. armigera*. The larvae were topically treated with a 0.5 µl of PEEF applied on the anterior pronotum of the insect. There were 3 different groups: the untreated larvae, the larvae topically treated with 1% (V/V) of PEEF and larvae treated with 2% (V/V) of PEEF. The feeding toxicity of the PEEF was tested on the early 2nd and the 4th instar larvae of *H. armigera.* The ingestion toxicity was assessed by incorporating the PEEF into the artificial diet. After this, the medium presented to the larvae in small Petri dishes. There were 4 different treatments: the control larvae fed on non-treated discs, the starved larvae, larvae fed with 1% PEEF (V/V) and larvae fed with 2% PEEF (V/V).

The deterrent effect of the PEEF was tested on neonate larvae based on a dual choice test. It consisted of releasing 15 first instar larvae of *H. armigera* in the Petri dish at a distance of 6 cm from two diet discs. Larvae were left to make a choice and counted on each disc at 1h, 8h and 24h after release. There were 2 different pairings: C *vs.* 1% and C *vs.* 2% with 15 Petri dishes for each choice test.

The pupation success was also assessed after feeding on PEEF treated diet. The pupicidal effect of two different concentrations of PEEF was tested on the 4th and 5th instar. Larvae were divided into 2 different independent groups with different dietary regimes: the

first group was allowed to feed freely on the treated diet from the 4th instar larvae until pupation while the second group was fed with the treated diet from the 5th instar until pupation. At the end of the bioassay, pupae were collected, counted and left in controlled conditions for hatching.

The topical application of the PEEF on the cuticle of the 2nd and 4th instar larvae of *H. armigera* caused a non-significant mortality. The survivorship of the 2nd instar larvae was not significantly different between treatments (Chi-square test, $\chi 2 = 10.97$, d.f. = 2, p>0.05). About 80% of the larvae survived after application of 0.5 µl of 2% PEEF on the anterior pronotum (Tukey´s HSD; p=0.08). The topical application of the PEEF on the 4th instar larvae showed non-significant effect (Chi-square test, $\chi 2 = 2.2$, d.f. = 2, p>0.05). Only one L_4 larvae was dead after treatment with 2% of PEEF.

The choice feeding test showed a significant difference between treatments 1 hour after releasing the larvae ($F_{2,42}=165.2$, p<0.001). At this time of observation, 45.77% of the 1st instar larvae were recorded on the control artificial medium while 26.66% were observed on the medium treated with 1% of PEEF. After 24h, more larvae moved from the treated to the untreated diet. An overall significant difference was observed ($F_{2,42}=165.2$, p<0.001) between treatments. At 2% concentration of PEEF, a significant difference between treatments was observed after 1 hour ($F_{2,42}=111.3$, p<0.001). After 24 hours, the larval presence on the control artificial medium was significant and less larvae were recorded on the treated diet in comparison with the previous times of observation (Tukey´s HSD; p <0.001).

After feeding on the artificial medium mixed with 2% of PEEF, the pupation rate of the 4th instar decreased significantly to 66% (Tukey´s HSD; p<0.05). However, there was no significant difference in comparison with the control after feeding from the 4th instar on a concentration of 1.25% of PEEF (Tukey´s HSD; p>0.05). About 76% of the larvae reached the pupation stage and 2 pupae did not hatch. When the larvae of *H. armigera* were fed on PEEF from the 5th instar larvae until pupation, a slight decrease of the pupation rate was observed. In this case, no significant effect was observed after administration of 1.25% (Tukey´s HSD; p>0.05) and 2% (Tukey´s HSD; p>0.05) of PEEF compared to the control where all larvae pupated.

After feeding on PEEF and seed oil treated medium, the larval weight gain differed significantly between treatments ($F_{2,33}=34.86$, p<0.001). Compared to the control, larvae fed

with 2% of PEEF showed a significantly lower weight gain (Tukey´s HSD; p<0.001). A significant difference was also observed when larvae were fed on 3% of seed oil in comparison with the control (Tukey´s HSD; p<0.05). Also, the ingestion of PEEF impacted significantly more the weight of larvae than the seed oil treatment (Tukey´s HSD; p<0.001). The pupal weight of *H. armigera* was significantly affected ($F_{2,33}$=4.32, p<0.05). Larvae fed with 2% of PEEF formed smaller pupae than the control treatment (Tukey´s HSD; p<0.05). However, no significant difference of the pupal weight was observed in the seed oil treatment in comparison with the control (Tukey´s HSD; p>0.05).

Although the limited killing effect of the extracts of *J. curcas* on *H. armigera*, the present study enlightened some insecticidal properties of these extracts that could be valorized to control this pest. The antifeedant effect of phorbol esters was the most important insecticidal aspect to be investigated and further applied in the management of *H. armigera*. This compound affected the assimilation of ingested food as well as the ability to convert ingested and digested food into growth. The pupicidal effect after feeding on PEEF treated diet would increase pupal mortality and decrease consequently the populations of *H. armigera* over generations. However, the application of *J. curcas* extracts as potential insecticidal compounds must be firstly subject to many toxicological studies before the concrete use as a standard bioinsecticide.

Keywords: *Jatropha curcas*, *Helicoverpa armigera,* Phorbol Esters Enriched Fraction, antifeedant, Lepidoptera, deterrent, food consumption.

1. Introduction

The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest widely distributed in Africa, Asia, Europe and South Pacific regions (Satyan *et al.,* 2009). It is considered as an economically important pest (Mensah *et al.,* 2015) of a wide range of plants in field (Mironidis *et al.,* 2008) and greenhouse crops (Sharma *et al.,* 2005). The major pest status of this insect derived from its polyphagy, high fecundity, its mobility, and seasonal migration (Feng *et al.,* 2005). These features reinforce the flexibility and plasticity of *H. armigera* to adapt to artificial and natural agro-ecosystems (CABI, 2016). *H. armigera* is listed as an A2 quarantine pest by EPPO (OEPP/EPPO, 2003). This quarantine status arises from the risk of introduction into glasshouse crops in northern Europe (CABI, 2016).

H. armigera is considered a polyphagous pest. It infests at least 60 cultivated and 67 wild host plants from many families including Asteraceae, Fabaceae, Malvaceae, Poaceae and Solanaceae (Pogue *et al.,* 2004). Damages spans from the vegetative growth phase through the flowering phase to the fructification period. Early instars of *H. armigera* are voracious leaf feeders that shift later to the developing bods, fruits, or bolls, leading to serious reduction in yield (CABI, 2016). During development, larvae may feed on many fruits and form inside the infested fruits a messy, watery, internal cavity filled with cast skins and feces. *H. armigera* is a key pest in Africa, Asia, Europe and the former USSR, and Oceania. In India, where *H. armigera* commonly destroys over half the yield of pulse crops, pigeon pea and chickpea, losses were estimated at over $US 300 million per annum (Reed *et al.,* 1982). In the late 1980s, losses of both pulses and cotton were estimated to exceed $US 500 million. In Australia, total losses were estimated Australian losses at $A 23.5 million (Wilson, 1982).

The adults of *H. armigera* tend to lay eggs during flowering phase on the upper third of healthy plants. Eggs can be found on vigorously growing terminals, on flowering parts but rarely on fruits. At optimal temperature, the larvae can hatch after three days (Liu *et al.,* 2004). The neonate larva eats through the eggs hell to emerge. In the beginning, larvae graze on tender young leaves, before moving to feed on buds, flowers, young pods, bolls or fruits (Kakimoto *et al.,* 2003). The larval development includes six growth instars and takes two to three weeks in summer or four to six weeks in spring or autumn. At the end of their development, fully grown larvae crawl to the base of the host plant, tunnel up to 10 cm into

the soil and form a chamber in which they pupate (Mironidis *et al.,* 2008). The duration of pupation depends on temperature, taking around two weeks in summer and up to six weeks in spring and autumn (CABI, 2016). *H. armigera* overwinters as a pupa, under the soil surface. Larval food quality influences the over-wintering readiness of the cotton bollworm (Liu *et al.,* 2007).

Because of the serious economic damages, chemicals have been heavily employed on *H. armigera* (Pogue *et al.,* 2004). As a result, populations of the cotton bollworm have developed resistance to several groups of active ingredients (Kranthi *et al.* 2002) and insecticides become less effective against this pest. However, botanical pesticides, a good alternative to chemicals, tend to have broad-spectrum activity and are specific in their mode of action and relatively safe to living organisms and environment (Baskar *et al.,* 2012). Some plants are rich with phytochemicals mobilized against pests and involved in plant defense (Richards *et al.,* 2015). These phytochemicals do not always induce the instant death of insect but affects the metabolic pathways and physiological functions (Prakash *et al.,* 1997, Prakash *et al.,* 2008). Such effects are the antifeedant, hormonal disruption and anti-oviposition effect. Therefore, plant secondary metabolites can be an alternative control method to chemicals.

Various botanical extracts have been reported to have a wide spectrum of toxicity against pests and have been long used as effective as synthetic chemicals for pest management. These plant-based compounds affect arthropod pests, vectors and other pathogens, but only few of botanicals are used in agriculture (Koul *et al.,* 2008) such as the physic nut tree *Jatropha curcas* well known for its toxicity. The genus *Jatropha* is native to tropical America and widely distributed in the tropical and subtropical areas of South America, Central America, Asia, Australia and Africa (Gübitz *et al.,* 1999). It belongs to the tribe Jatropheae, orders Malpighiale in the Euphorbiaceae family and contains more than 170 known species (Wurdack, 2008). The most widespread specie around the world is *Jatropha curcas* (Ratnadass *et al.,* 2012).

The aim of the present study was to assess the insecticidal properties of the physic nut tree *J. curcas* on the cotton bollworm *H. armigera*. We selected the Phorbol Esters Enriched Fraction (PEEF) and the seed oil (SOi) as extracts of *J. curcas* to evaluate their efficacy on *H. armigera*. The bioassays have concerned the larval stages as well as the adults

in laboratory and greenhouse conditions. The experiments were set-up with plants as well as with artificial diet. We addressed the following questions:

- Does the PEEF of *J. curcas* induce larval mortality by topical application?

- Does PEEF-treated diet affect the larval feeding behavior?

- Which larval stage is the most susceptible to the toxicity of PEEF?

- Does feeding on PEEF show any pupicidal effect on *H. armigera*?

2. Material and methods

2.1. Insect culture

The larvae of *H. armigera* used in this bioassay were collected from a laboratory culture. This rearing was originally established from eggs purchased from Bayer Crop Science AG (Monheim, Germany). Eggs were incubated for 4 days in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) under controlled conditions (20 ±1°C temperature, 65% relative humidity and 16L: 8D photoperiod). Neonate larvae were reared in groups of 150 larvae in Petri dishes (86.5 mm diameter x 14.5 mm height) (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany) filled with 20 g of artificial medium. At the beginning of the 2nd instar larvae, caterpillars were separated to be reared individually in small Petri dishes (35 mm x 10 mm) and kept in sterile boxes (10 cm x 10 cm) (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany) in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) under controlled conditions (22 ±1°C temperature, 65% relative humidity and 16L: 8D photoperiod). At the end of the life cycle, pupae were separated into females and males then placed at a ratio of 2:2 in sterile plastic boxes (18cm length × 13cm width ×5.5cm height) covered with a perforated lid. Adults were supplied with a honey solution (1% W/V) mixed with 2g of pollen as food resource.

2.2. Host plant

The greenhouse experiments were conducted on 3 weeks old tomato plants. Tomato plants (*Solanum lycopersicum* variety: Rio Grande; Reinsaat® KG, Sankt Leonhard am Hornerwald, Austria) were grown in plastic trays from seeds in a greenhouse chamber (22 ±3°C temperature, 60% relative humidity and 16L: 8D photoperiod). After 10 days, seedlings were transplanted into 11 cm diameter pots filled with non-sterile soil (Fruhstorfer Erde Typ 25, Hawita Gruppe GmbH, Vechta, Germany, peat fine structure with volcanic clay, pH: 5.7-

6.3, Fertilization of 200-300mg N) and sand mixture (3:1 volume). Plants were grown in the chamber, regularly irrigated and fertilized once a week since the 3 week after potting (fertilizer: Hakaphos 2g/L (15% N, 11% P2O5, 15% K2O, 1% Mg, 0.1% Fe, 0.1% Mn, 0.04% Cu, 0.025 %B and 0.005 % Mo)).

2.3. Artificial medium

The laboratory bioassays such as the antifeedant effect of *Jatropha* were tested by mixing these extracts with the insect diet. Larvae were also reared on artificial diet for mass rearing. The artificial medium is mainly composed of bean powder and wheat germ mixed with yeast extract as a phagostimulant.

2.4. Jatropha bioinsecticides

In this study, two different extracts of *J. curcas* were screened for their insecticidal effect: the seed oil (SOi) provided by the company of JatroSolutions GmbH (Stuttgart, Germany) and the PEEF extracted in the laboratories of P.P.M e.V. (Pilot Pflanzenoeltechnologie Magdeburg, Germany). They have been extracted and purified following the method of Devappa *et al.* (2010).

2.5. Experimental bioassays

2.5.1. Contact toxicity of PEEF

The contact toxicity of PEEF was tested on the 2nd and 4th instar larvae of *H. armigera*. Larvae were collected from a stock colony maintained in a climatic chamber (WB 750 KFL; Mytron Bio-Und Solartechnik GmbH, Germany) under controlled conditions (22 ±1 °C temperature, 65% relative humidity and 16L: 8D photoperiod). The larvae were topically treated with 0.5 µl of PEEF applied with a micropipette on the anterior pronotum of the insect. There were 3 different groups in this bioassay for each larval instar: the untreated larvae, the larvae topically treated with 1% (V/V) of PEEF and larvae treated with 2% (V/V) of PEEF. After treatment, larvae were released in small Petri dishes (35 mm x 10 mm) (Sarstedt, Aktiengesellschaft & CO Nuembrecht, Germany) filled with 4.5 g of diet. The mortality was daily recorded until the 8th day post treatment. Larvae were considered dead when no reaction to the stimulation was observed. There were 15 larvae per treatment and the bioassay was set-up once. Larvae were maintained in a climatic chamber in controlled conditions (22 ±1°C temperature, 65% relative humidity and 16L: 8D photoperiod).

2.5.2. Deterrent effect of the PEEF

This choice test was conducted in in vitro conditions on the 1st instar larvae of *H. armigera*. After cooling down to room temperature, the artificial medium was cut into small discs of 1 g weight with a clean borer. The control diet discs were immersed in bidest water whereas the treated diet disc were dipped in a solution of 1 % (V/V) and 2% (V/V) of PEEF contained in a 50 ml falcon tube. The discs were left overnight dipped in the solutions in a cold room at 4ºC. In the day of the set-up of the bioassay, discs were taken out of the tubes and placed on sterile filter papers to dry. After drying, 2 different discs were placed in a 14 cm Petri dish lined with a sterile filter paper (125 mm diameter, Cat Nº 1001 125) (Schleicher and Schuell Bioscience GmbH, Dasse, Germany). The control and treated discs were separated by a distance of 9 cm. The dual choice test consisted of releasing 15 first instar larvae of *H. armigera* in the Petri dish at a distance of 6 cm from the diet discs. The larvae were left to make a choice and the Petri dishes were sealed with Parafilm to avoid the dryness of the food discs. Larvae were counted on each disc at 1h, 8h and 24h after release. There were 2 different pairings: C *vs.* 1% (V/V) and C *vs.* 2% (V/V) with 15 Petri dishes for each choice test. The Petri dishes were randomly distributed and kept in a climatic chamber under controlled conditions (22 ±1 ∘C, 16:8 h (L: D) and 65% RH).

2.5.3. Pupicidal effect of PEEF

The pupation success of the larvae of *H. armigera* was assessed after feeding on PEEF treated diet. In this experiment, the pupicidal effect of two different concentrations of PEEF was tested on the 4th and 5th instar. Larvae were divided into 2 different independent groups with different dietary regimes: the first group was allowed to feed freely on the treated diet from the 4th instar larvae until pupation, while the second group was fed with the treated diet from the 5th instar until pupation. For that, larvae were firstly reared together after hatching, in groups of 40 in Petri dishes containing 20 g of untreated diet. In order to avoid cannibalism, larvae were separated at the end of the 2nd instar and reared individually in small Petri dishes filled with 4.5 g of medium. In order to set up the bioassay with age-synchronized insects, only larvae that molted simultaneously at the 4th instar for the first group and the 5th instar for the second group were used.

To avoid the degradation of the phorbol esters, the medium was treated with the same immersion method as described in the previous experiments. There were 3 different treatments for each group: larvae fed on untreated diet, larvae fed on diet treated with

1.25% (V/V) of PEEF and larvae exposed to diet treated with 2% (V/V) of PEEF. Larvae were gently weighed and individually transferred with forceps to 90 mm Petri dishes lined with a sterile filter paper. The artificial medium was daily changed for every treatment until pupation. At the end of the bioassay, pupae were collected, counted, measured and left in controlled conditions for hatching. There were 12 Petri dishes per treatment randomly distributed in a climatic chamber (22 ±1 ∘C, 16:8 h (L: D) and 65% RH).

2.5.4. Effect of the PEEF on the food consumption

The antifeedant effect of *J. curcas* compound was assessed on the 3rd instar larvae of *H. armigera* under choice and non-choice conditions. The artificial medium was mixed with a concentration of 2% (V/V) of PEEF and presented to the larvae in form of food discs of 1.3 g weight cut with a sterile borer. The food was left to dry in laboratory conditions on a clean surface then placed with forceps separated by 5 cm in a 14 cm Petri dish on a moistened filter paper (125 mm diameter, Cat № 1001 125) (Schleicher and Schuell Bioscience GmbH, Dasse, Germany).

Larvae used in this bioassay were collected after hatching from a stock culture and reared on untreated artificial medium. They were separated at the end of the 2nd instar until they reached the 3rd instar larvae. The molting skin was used as an indicator of the insect development. We used larvae having freshly and simultaneously molted and with similar weights. In the choice test, one larva per Petri dish was exposed to 2 different food discs: the control and the 2% tread medium. Larvae were released at 5 cm distance from the food resource and left to make a choice before starting feeding on the chosen diet. There were 15 sealed Petri dishes each with only one larva individually feeding.

In the non-choice assay, only one food disc was presented to the larva, either the control food discs or the diet treated with 2% of PEEF. There were 15 larvae per treatment released separately in 15 Petri dishes. In both bioassays, larvae were left to feed freely during 3 days on the diet. At the end of the experiment, larvae were removed and the food discs were weighed to deduce the consumed amount of diet. The two bioassays were conducted in a climatic chamber under controlled conditions (22 ±1 ∘C, 16:8 h (L: D) and 65% RH).

2.5.5. Comparison of the antifeedant effect of PEEF and the seed oil

The effect of the seed oil and the PEEF on the growth of *H. armigera* was assessed under laboratory conditions. The 4th instar larvae were selected in this feeding test. They have a relative steadily feeding activity and release weighable pellets of feces. Larvae used in this bioassay were originating from eggs laid by the same female. They were reared in group of 40 larvae during the two first instars. At the 3rd instar, when the cannibalism occurred, caterpillars were separated and reared individually in small Petri dishes (35 mm x 10 mm) on untreated diet. At the end of the 3rd instar, newly molted larvae to the 4th instar were selected. They were weighed and placed individually in sterile Petri dishes (86.5 mm diameter x 14.5 mm height) lined with a filter paper and sealed after releasing the larva. Insects were allowed to feed on artificial medium until the 6th instar larvae. There were 3 different treatments: control, diet mixed with 2% (V/V) PEEF and diet treated with 3% (V/V) of seed oil. At the end of the bioassay, feces, larvae and pupae were weighed. There were 12 larvae per treatment feeding individually in Petri dishes. The bioassay was conducted in a climatic chamber under controlled conditions (22 ±1 ∘C, 16:8 h (L: D) and 65% RH).

2.6 Data analysis

Statistical processing of results was carried out by standard methods using the statistical software R (v 3.0.2) (R Development Core Team, 2008). Prior to analysis, all data (mortality, weight and food consumption) were tested for homogeneity of variance and normality. To detect the difference between treatments, we performed One-way analysis of variance (ANOVA). If significant differences between groups were detected, means were compared by performing Tukey's test at $\alpha = 0.05$.

The mortality of larvae was analyzed using generalized linear models (GLM) taking account of the binomial distribution of the data (alive or dead). To correct the anomalies in data variation (overdispersion/underdispersion), we proceeded to the quasi-binomial distribution error in data analysis. Thereafter, Tukey's test at $\alpha = 0.05$ was employed to detect differences between means.

Giving that some larvae did not make a choice during the feeding choice test, the non-choice category was also considered during the data analysis which made counts distributed into 3 major groups (Control, treated and no-choice). This non-binary aspect of data deriving from choice bioassays (Control, Treated or No-choice) made it unsuitable to perform a binomial test for data analysis. In this case, we proceeded to generalized linear

models (GLM) using Poisson distribution followed by quasi-poisson models analysis in case of dispersion anomalies. In case of significant differences between groups, data were subject to a Tukey´s test at α = 0.05 to separate the means. The data of food consumption obtained from choice feeding test were analyzed based on Student´s test to compare the amount of diet consumed between two groups.

3. Results

3.1. Contact toxicity of PEEF

The topical application of the PEEF on the cuticle of the 2nd and 4th instar larvae of *H. armigera* caused a non-significant larval mortality. The survivorship of the 2nd instar larvae was not significantly different between treatments (Chi-square test, $\chi 2 = 10.97$, d.f. = 2, p>0.05). About 80% of the larvae survived after application of 0.5 μl of 2% PEEF on the anterior pronotum (Tukey´s HSD; p>0.05). The application of 1% of PEEF did not cause a significant mortality in comparison with the control and a similar number of larvae survived compared to the control. PEEF induced a late toxic effect on the larvae of *H. armigera*. In fact, no mortality was recorded during the first seven days of the bioassay. The topical application of the PEEF on the 4th instar larvae showed non-significant effect (Chi-square test, $\chi 2 = 2.2$, d.f. = 2, p=0.33). Only one larva was dead after treatment with 2% of PEEF. The dead larvae showed symptoms of necrosis which spread from the application point to the whole cuticle of the insect. All the surviving larvae in all treatments continued their development to reach the pupal stage. All pupae hatched and gave healthy flying adults. We observed no larval mortality in the control groups.

3.2. Deterrent effect of the PEEF

This choice test showed a significant difference between treatments 1 hour after releasing the larvae ($F_{2,42}=165.2$, p<0.001; Figure 1a). At this time of observation, 45.77% of the 1st instar larvae were recorded on the control artificial medium while 26.66% were observed on the medium treated with 1% of PEEF. We recorded 27% of the larvae that did not make a choice. There was a significant difference between the control and the PEEF based treatment (Tukey´s HSD; p<0.001). After 8 hours, a significant difference was observed between treatments ($F_{2,42}=188.4$, p<0.001). The number of larvae that did not make choice was similar to the previous observation. However, the number of larvae present on the PEEF treated diet decreased to 19% but increased in the other hand on the control diet to 52.88%

(Tukey´s HSD; p <0.001). After 24h, more larvae moved from the treated to the untreated diet. An overall significant difference was observed ($F_{2,42}$=165.2, p<0.001) between treatments. About 73% of larvae were recorded on the control artificial medium however 13% of the larvae were observed on the diet mixed with PEEF (Tukey´s HSD; p <0.001).

At 2% concentration of PEEF, a significant difference between treatments was observed after 1 hour ($F_{2,42}$=111.3, p<0.001; Figure 1b). We recorded 50% of larvae on the control medium while 26.66% of larvae were observed on the diet treated with PEEF (Tukey´s HSD; p <0.001). There were 22% of larvae did not make a clear choice during this time. After 8 hours from the set-up of the experiment, a significant difference was detected between treatments ($F_{2,42}$=207.4, p<0.001). The number of larvae that choose to feed on the control diet increased to 55% while the number of larvae on the PEEF treated diet dropped to 17.61% (Tukey´s HSD; p <0.001). The number of larvae that did not make a choice remained constant. After 24 hours, the larval presence on the control artificial medium became more significant and less larvae were recorded on the treated diet in comparison with the previous time of observation (Tukey´s HSD; p <0.001). We recoded 69.52% first instar larvae on the untreated diet whereas only 10% of the larvae were observed on the PEEF based medium.

3.3. Pupicidal effect of PEEF

The ingestion of PEEF by larvae from the 4th instar showed a significant difference between treatments on the pupation success ($F_{2,33}$=6.67, p<0.001). After feeding on the artificial medium mixed with 2% of PEEF, the pupation rate of the 4th instar decreased significantly to 66% (Tukey´s HSD; p<0.05). However, there was no significant difference in comparison with the control after feeding from the 4th instar on a concentration of 1.25% of PEEF (Tukey´s HSD; p=0.06). About 76% of the larvae reached the pupation stage and 2 pupae did not hatch.

When the larvae of *H. armigera* were fed on PEEF from the 5th instar larvae until pupation, a slight decrease of the pupation rate was observed. In this case, no significant effect was observed after administration of 1.25% (Tukey´s HSD; p=0.83) and 2% (Tukey´s HSD; p=0.59) of PEEF compared to the control where all larvae pupated. We recorded respectively after ingestion of 1.25% and 2% of PEEF 94.44% and 91.66% pupation success and we observed healthy adults.

The pupal size of larvae treated from the 4th instar was significantly different between treatments ($F_{2,85}$=3.12, p<0.05). The pupae collected from larvae fed with 2% of PEEF were significantly smaller than the control (Tukey´s HSD; p<0.05) while no effect on the pupal size was detected after feeding on 1.25% of PEEF (Tukey´s HSD; p=0.43). The control pupa measured 19.44 ±0.69 mm, the pupae obtained from larvae fed with 1.25% of PEEF measured 19.21 ±0.83 mm and the larvae exposed to 2% had a size of 18.95 ±0.69 mm. There was no statistical difference on the length of pupae when larvae were exposed to the PEEF from the 5th instar larvae ($F_{2,100}$=1.22, p=0.29). The pupal size was for the control, 1.25% and 2% treatment respectively 19.63 ±0.59 mm, 19.47 ±0.82 mm and 19.36 ±0.78 mm. Larvae that failed to pupate had a thin cuticle with mainly pupal distortions with subsequent wing malformations of the adults.

3.4. Effect of the PEEF on the food consumption

In non-choice conditions, the 2nd instar of *H. armigera* consumed significantly high amounts of the untreated medium in comparison with larvae exposed to the PEEF treated medium (Student's t-test, d.f. = 60.89, p<0.001). The final weight of the medium was 375.2 ±07 mg and 891.4 ± 11 mg respectively in the control and PEEF treated medium. The larvae consumed 879.2 ±6 mg from the untreated diet and only 362.2 ±14 mg from the medium treated with the *J. curcas* compound (Figure 2). In choice conditions, when the control and PEEF-treated food discs were presented together to larvae, the consumed amount from the treated diet decreased to 30.9 ±2 mg. Larvae preferred the untreated diet, fed more on the control artificial medium and avoided to feed on the treated food discs (Student's t-test, d.f. = 45.75, p<0.001).

3.5. Comparative study of the insecticidal effect of the seed oil and the PEEF

The weight gain differed significantly between treatments ($F_{2,33}$=34.86, p<0.001; Figure 3). Compared to the control, larvae fed with 2% of PEEF from the 4th to 6th larval instar showed a significantly lower weight gain (Tukey´s HSD; p<0.001). A significant difference was also observed when larvae were fed on 3% of seed oil in comparison with the control (Tukey´s HSD; p<0.05). The results showed also that the ingestion of PEEF impacted significantly more the weight of larvae than the seed oil treatment (Tukey´s HSD; p<0.001).

The pupal weight of *H. armigera* was significantly affected ($F_{2,33}$=4.32, p<0.05). Larvae fed with 2% of PEEF formed smaller pupae than the control treatment (Tukey´s HSD;

p<0.05). However, no significant difference of the pupal weight was observed in the seed oil treatment in comparison with the control (Tukey´s HSD; p=0.81). In the control, seed oil and PEEF pupae weighed respectively 345.8 ±7 mg, 337.3 ±6 mg and 306.8 ±14 mg. There was no significant difference between the pupal weight of the larvae fed on seed oil and larvae fed on PEEF (Tukey´s HSD; p=0.08).

The weight of feces produced by the larvae was significantly different between treatments ($F_{2,33}$=24.91, p<0.001). Larvae exposed to the food treated with 2% of PEEF produced more feces in comparison with the control (Tukey´s HSD; p<0.001). Moreover, larvae fed on seed oil-admixed diet showed a significant difference in terms of feces production to the control (Tukey´s HSD; p<0.05). There was a significant difference between the seed oil and PEEF treatment (Tukey´s HSD; p<0.05).

4. Discussion

4.1. Contact toxicity of PEEF

The topical application of the PEEF caused low mortality on the 2nd instar larvae and almost no visible effect on the 4th instar. Our findings confirmed the results obtained by Ratnadass *et al.* (2009) and showed a lower toxicity of PEs by contact application than by ingestion. Although the skin irritant effect of phorbol has been early proved by Schmidt *et al.* (1980), our bioassay showed a weak evidence of this compound as an insecticide applied topically on *H. armigera*. However, the few dead larvae showed signs of cuticle inflammation and epidermal hyperproliferation. These symptoms were reported by Goel *et al.* (2007) and resulted from hypersynthesis of DNA leading to the necrosis of the cuticle (Rios *et al.,* 2006).

Many plants of the family of *Euphorbiacea* are known to contain oil, soap or latex possessing marked skin and mucous membrane irritant properties. The compounds involved in these effects have been shown to be esters of polyfunctional diterpene alcohols (Schmidt *et al.,* 1980). De Vry *et al.* (2005) reported that the PEs fraction induced a dermatitis and cuticle irritation when topically applied on the epidermal tissues, resulting in cell damages and cuticle thickness. Declos *et al.* (1980) showed that phorbol esters were able to bind effectively to cuticle receptors, induce inflammation and tissue damage.

The necrosis and cell darkening, observed on the cuticle of the 2nd instar larvae, were also reported by Najar-Rodríguez *et al.* (2007). After topical application, the lipophilic and oily nature of PEEF helps to penetrate the cell, accumulate inside the cytoplasm and affect

their structural and functional properties. The seed oil of *J. curcas* was also reported as a cuticle irritating compound (Sabandar *et al.,* 2013) and had been shown to cause cell disintegration and necrosis followed by a general burn effect of the insect cuticle (Devappa *et al.,* 2011). Although there is evidence of PEs as a toxic compound, a weak contact toxicity was proven in this bioassay.

4.2. Deterrent effect of the PEEF

A feeding deterrent is a compound that prevents feeding without killing directly the insect which remains near the treated foliage or substrate and dies through starvation (Packiam *et al.,* 2014). This feeding choice test assessed how effectively the PEEF inhibited insect feeding and proved the deterrent effect particularly on the 1st instar larvae of *H. armigera*. At a concentration of 1% and 2% of PEEF, more larvae were recorded feeding on the control artificial medium, few were observed temporarily around the PEEF treated diet.

Most effective antifeedants on insects are sesquiterpene lactones, diterpinoids, triterpinoids, quinoline and indole alkaloids, which are presented in plants (Baskar *et al.,* 2009). The photochemistry of the family Euphorbiaceae and the genus *Jatropha* was reviewed by Devappa *et al.* (2011) and revealed that the predominant ingredient is phorbol ester, providing a broad array of defenses against arthropod herbivores (Kumar *et al.,* 2012). The deterrent effect of the PEEF used in our bioassay was mainly ascribed to the PEs, a diterpinoid fraction contained in the seed oil of *J. curcas*. The feeding inhibition effect of this compound was investigated by Devappa *et al.* (2012) on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The results showed that the dietary intake by *S. frugiperda* was severely affected when fed on corn leaves treated with 0.0625 and 0.125 mg PEs ml^{-1} (w/v) of PEEF.

A potential rejection of food may act via various mechanisms: olfactory repellency inducing the rejection of the substrate without coming in contact with the food; true gustatory deterrence after tasting the substrate and the larvae cause some small biting points on the substrate; physical obstacle via oral mechanoreceptors and/or toxicity via post-ingestive response, in this case, the larvae will consume relatively much food before they reject them (Li *et al.,* 2014). In our bioassay, the gustatory deterrence could explain our results since most of the larvae came shortly in contact with the PEEF treated medium before moving few hours after to the untreated food. This observation has already been reported by Carpinella *et al.* (2003) after testing the deterrent effect of a limonoid from

Melia azedarach (Meliaceae) on the larvae of the beetle *Epilachna paenulata* (Coleoptera: Coccinellidae).

Our findings suggested that the PEEF elicited an immediate rejection of the treated food on the 1st instar larvae of *H. armigera*. It could be inducing a primary antifeedant effect, probably via the gustatory pathway regulated by sensory organs of the mouth parts (Wheeler *et al.,* 2001, Isman *et al.,* 2006). Many plant extracts have been reported to induce a feeding inhibition effect on the cotton bollworm *H. armigera*. Kathuria *et al.* (2005) showed that crude leaf extracts of *Eucalyptus camaldulensis* (Myrtaceae) and *Tylophora indica* (Asclepiadaceae) caused a significant feeding deterrence when exposed to the larvae of *H. armigera* on cabbage leaf discs. Baskar *et al.* (2012) reported that ononitol monohydrate isolated from the Sickle Senna plant *Cassia tora* (Fabaceae) showed at 1000 ppm more than 65% antifeedant activity against *H. armigera* and *Spodptera litura* (Lepidoptera: Noctuidae) when larvae feed respectively on fresh treated cotton leaves and castor leaves.

4.3. Pupicidal effect of PEEF

The effect of botanical compounds on the pupal formation of *H. armigera* showed in our bioassay has been so far mentioned in many studies on Lepidoptera. Baskar *et al.* (2009) reported that the ingestion of crude hexane extract of *Atalantia monophylla* (Rutaceae) at a concentration of 100 ppm, 250 ppm, 500ppm and 1000 ppm induced different aspects of malformations (larval–pupal intermediate, pupal and adult intermediate) on *H. armigera*. Baskar *et al.* (2012) showed also that feeding on leaves dipped in 125, 250, 500 and 1000 ppm of ononitol monohydrate extracted from the plant *Cassia tora* exhibited pupicidal activity against *H. armigera* and *S. litura* (Lepidoptera: Noctuidae). Moreover, Baskar *et al.* (2014) reported that triterpenoid friedelin isolated from hexane extract of *Azima tetracantha* leaves induced a high pupicidal effect at a concentration of 130.47 and 226.41 ppm on *H. armigera and S. litura* after feeding respectively on castor and fresh cotton leaves.

It was observed in our bioassay that the pupal size of larvae fed on 2% of PEEF from the 4th larval stage until pupation differed significantly from the control. Pupae were smaller than those obtained from larvae fed on untreated diet. A similar observation was reported by Koul *et al.* (2004) on *H. armigera* and showed that the aglaroxin A extracted from *Aglaia elaeagnoidea* (Meliaceae) caused a strong antifeedant effect and affected the larval growth.

Subsequently, the pupal duration was prolonged and the pupae were smaller in size both in *H. armigera* and *S. litura*. In the same way, Abdelgaleil *et al.* (2005) reported that methyl 6-hydorxyangolensate and 7-dideacetylkhivorin exhibited a significant reduction of adult emergence and hatching when mixed with diet of *S. littoralis*. In this study some adults emerged and were small in size with varied abnormalities.

The pupicidal effect of PEEF as a single active compound has not been reported in previous studies. However, Chauhan *et al.* (2015) showed that some extracts from *Jatropha* exhibited a pupicidal effect on the housefly, *Musca domestica* (Diptera). In this study, the variation in LC_{50} in hexane and ether extracts of *J. curcas* leaf was found to be 8.88 - 8.95 mg/cm^2. The LC_{50} of the hexane and ether extracts of *J. curcas* bark varied between 9.72 - 10.33 mg/cm^2. Also, the pupicidal effect of the root extract of *J. curcas* exhibited a LC_{50} of 13.10 mg/cm^2 in both hexane and ether solvent. Moreover, Bullangpoti *et al.* (2012) showed that feeding on diet mixed with ethanol extract from senescent leaves of *J. gossypifolia* caused larval mortality and pupal intermediates on *S. frugiperda* (Lepidoptera: Noctuidae). In our bioassay, pupal intermediates were frequently observed at a concentration of 2% of PEEF when larvae were fed from the 4th larval stage. Larvae that reached the pupal stage formed pupae that became dark black with absence of movement few days after pupation.

4.4. Effect of the PEEF on the food consumption

The results obtained in this bioassay confirmed the deterrent/antifeedant properties of the PEEF of *J. curcas*. Antifeedants were earlier defined by Isman (1990) as ``behavior-modifying substances that deter through a direct action on taste organs in insects´´. In our bioassay, the phorbol esters of *J. curcas* affected the food intake of the 2nd instar of *H. armigera* by exerting strong direct antifeedancy and larvae did not feed on the diet treated with PEEF.

According to our bioassay, the food intake was reduced in the non-choice test or even suppressed in the choice test, depending on the concentration of PEEF. The reduction of food consumption after treatment with *J. curcas* extracts has been mentioned by Acda (2009) on the Philippine milk termite *Coptotermes vastator* (Isoptera: Rhinotermitidae). Termites consumed less paper when it was mixed with a concentration of 5% (V/V) of the seed oil of *J. curcas*. Khani *et al.* (2012) reported that the seed oil of *J. curcas*, rich with PEs, induced an antifeedant effect against the rice moth *Corcyra cephalonica* (Lepidoptera: Pyralidae) which increased with increasing plant extract concentrations.

Moreover, Devappa *et al.* (2012) reported the antifeedant effect of the PEEF on the 3rd instar larvae of the fall armyworm *S. frugiperda* (Lepidoptera: Noctuidae), induced at small PEEF concentrations.

4.5. Comparative study of the insecticidal effect of the seed oil and the PEEF

Insect growth is directly correlated with nutrient input (Hwang *et al.,* 2008). In our study, the seed oil and the PEEF of *J. curcas* have been found to have clear antifeedant and toxic effects on the larvae of *H. armigera*. The experiment showed that the 4th instar larvae of cotton bollworm came in contact with the food treated with 3% of seed oil and the other treated with 2% of PEEF. The reduced feeding activity on PEEF treated food after exposure could be the consequence either of rejection of food without substantial ingestion or of rejection following short periods of feeding. No relevant larval gain of *H. armigera* was observed, in particular after exposure to the PEEF of *J. curcas*.

Botanical and plant based extracts could affect in Lepidoptera the assimilation of ingested food as well as the ability to convert ingested and digested food into growth. Sadek (2003) reported that after feeding the 6th instar larvae of the African cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) on the leaf extract of *Adhatoda vasica* (Acanthaceae), the larval growth was severely affected. Also, Martinez *et al.* (1999) showed that the ability to convert food into biomass was reduced when the 3rd instar larvae of *S. littoralis* larvae were fed on diet treated with Azadirachtin for two days. Martinez *et al.* (2001) hypothesized that, in the absence of an adequate supply of amino acids, other nutrients then in excess for growth might be diverted into other metabolic survival pathways.

The reductions in food intake and the decrease of the ability to convert food into biomass would eventually affect the pupal weight. The antifeedant properties of the extracts of *J. curcas* has been shown by Devappa *et al.* (2012) and Ribeiro *et al.* (2012) on the fall armyworm *S. frugiperda* (Lepidoptera: Noctuidae). However, no previous studies reported the effect of PEEF as a compound derived from *J. curcas* on the pupal weight of *H. armigera*. Nevertheless, Ribeiro *et al.* (2012) reported no significant changes in pupal weight after feeding larvae *S. frugiperda* from their neonate stage on the methanol leaf extract of *J. curcas*. Also, Bullangpoti *et al.* (2012) showed that the leaf extract of *J. gossypifolia* delayed the pupation time of 7 days and caused some pupal deformations at higher doses. The bioassay showed also that the larvae fed on 2% of PEEF produced more feces then the

control larvae. Chen *et al.* (2007) suggested that due to the poor efficiency of both ingested and digested food, mixed in our case with PEEF, much of the consumed food was not used and released as fecal matter.

5. Conclusion

Research so far published and focused on the genus *Jatropha spp.* and particularly the physic nut tree *J. curcas*, reported that extracts from this plant (leaves, seeds and oil) exhibited a variety of insecticidal properties. These effects on insects ranged from ingestion toxicity to anti oviposition deterrence to repellent effect and antifeedant activity. According to our study, the most relevant effect in terms of plant protection could be the antifeedant effect on early instar larvae. This feeding inhibition effect was mainly due to diterpene rich compounds, particularly phorbol esters. In spite of these insecticidal properties, the biocide characteristics of *J. curcas* are not yet valorized in terms of plant protection. The efficacy of the PEEF and the seed oil in reducing damage caused by the cotton bollworm would make these compounds a promising alternative for pest management. However, the lack of detailed toxicological studies and the absence of research investigating the potential side effects of phorbol esters limit their use as bioinsecticides in pest management programs.

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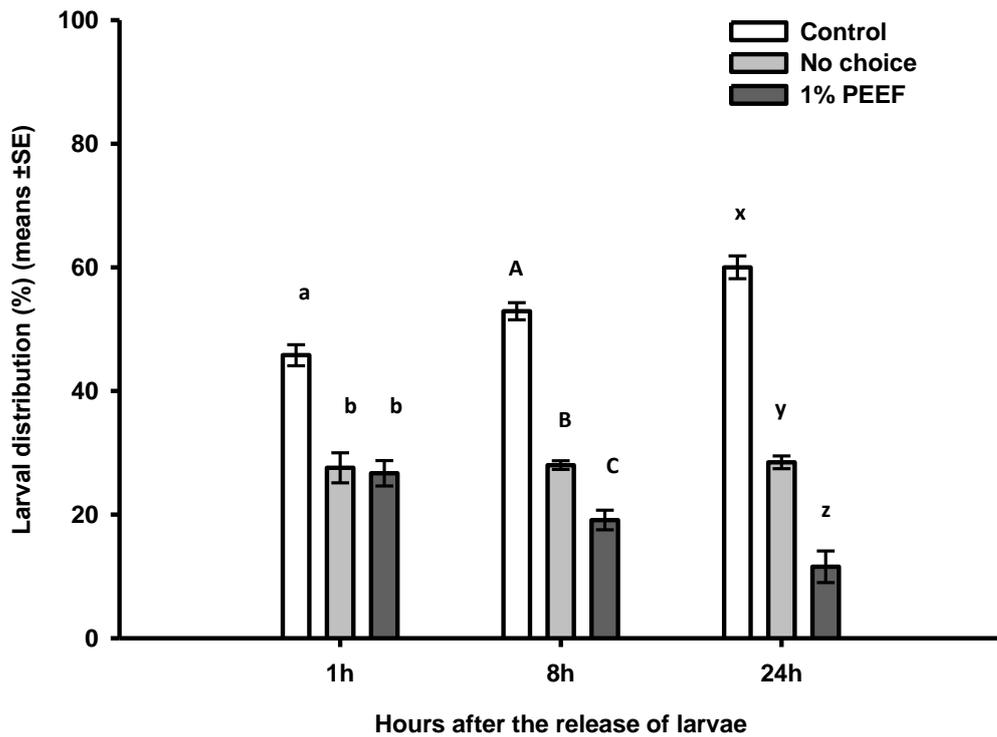
Figure 1a: Deterrent effect of PEEF on neonate larvae of *H. armigera*. Control (untreated diet) *vs.* 1% PEEF-treated diet. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
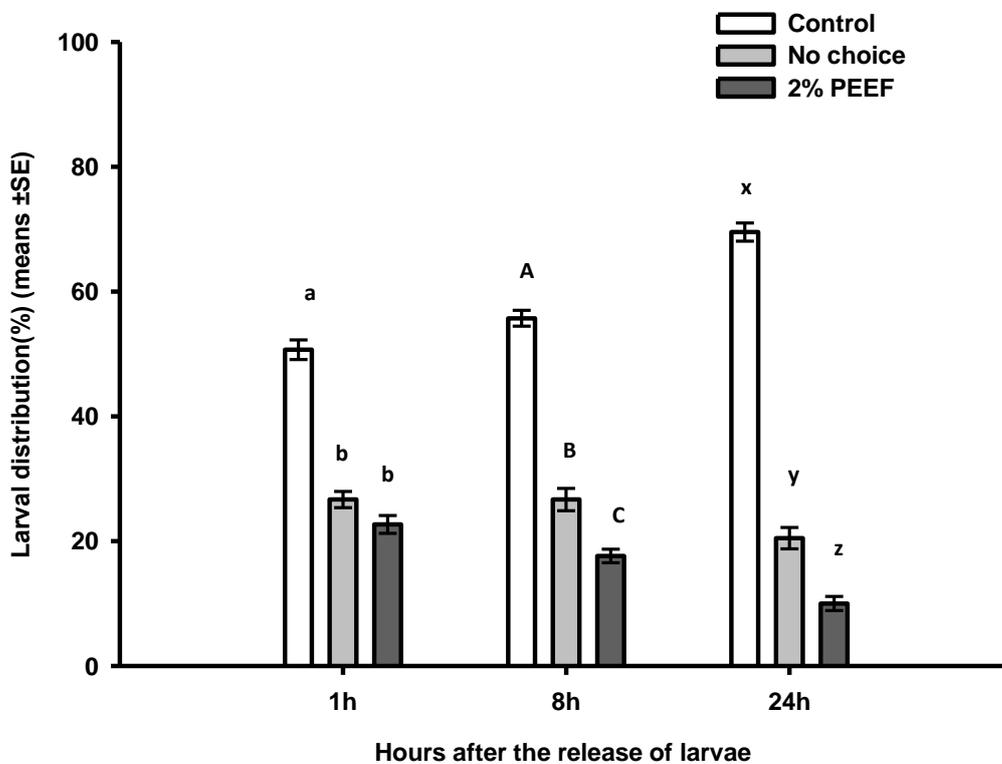
Figure 1b: Deterrent effect of PEEF on neonate larvae of *H. armigera*. Control (untreated diet) *vs.* 2% PEEF-treated diet. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, p<0.05).
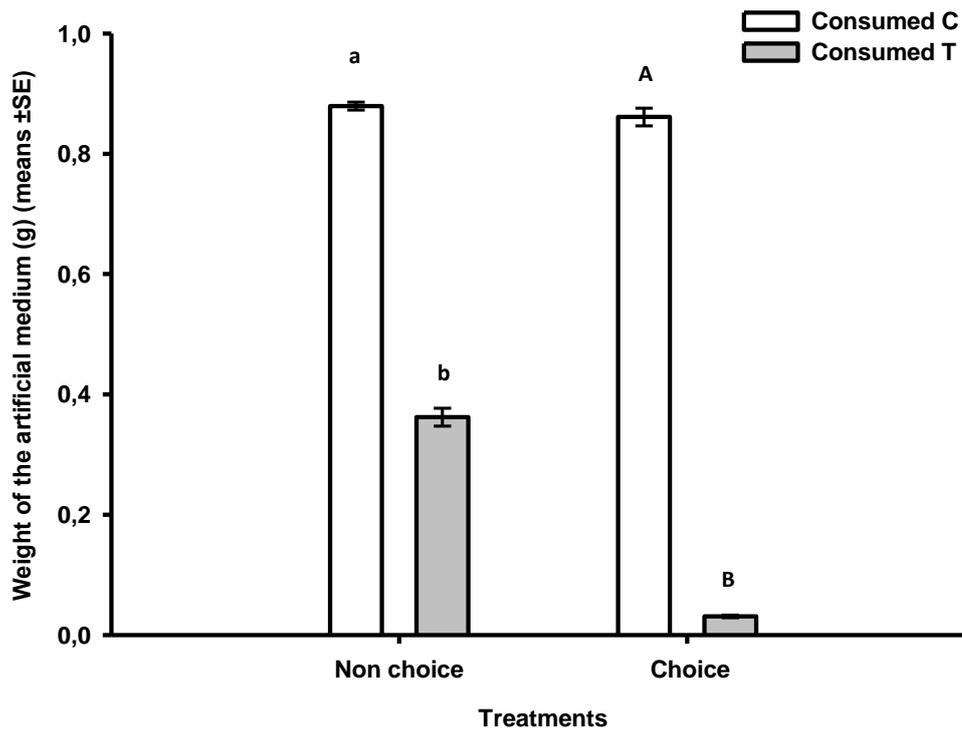
Figure 2: Effect of 2% PEEF on food consumption of 3rd instar larvae in choice and non-choice conditions. **Consumed C:** control diet. **Consumed T:** PEEF-treated diet. Bars denoted within the same group with different letters are significantly different (Student´s t-test, $p < 0.05$).
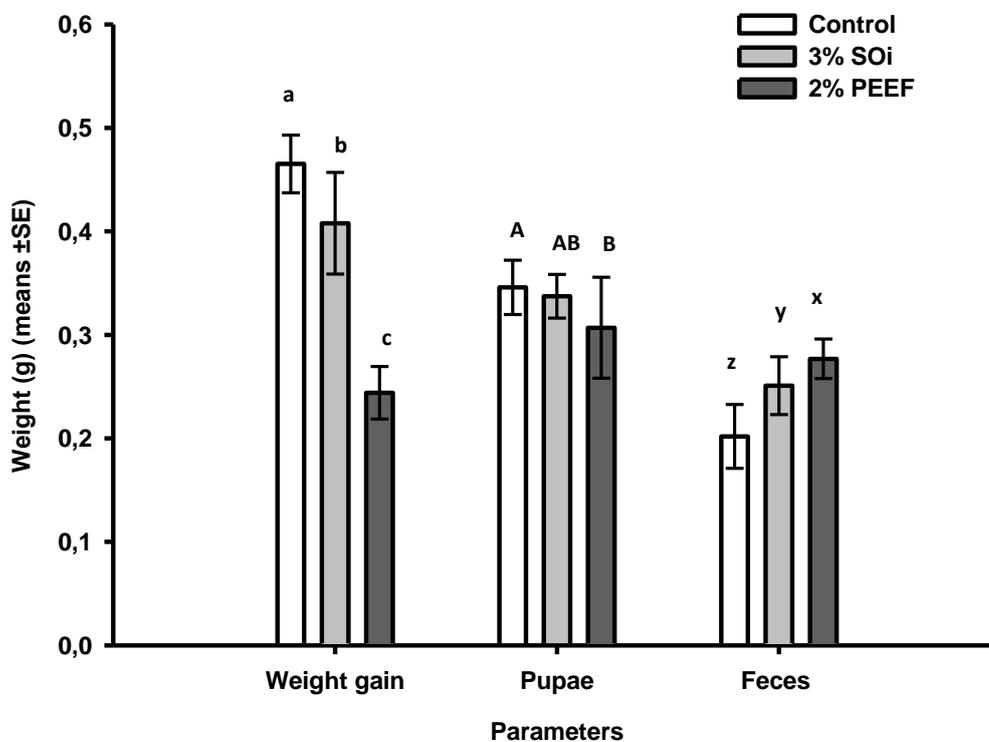
Figure 3: Effect of 3% of seed oil (SOi) and 2% of PEEF on the weight gain, pupal weight and feces production by larvae. Bars denoted within the same group with different letters are significantly different (All Pairwise Multiple Comparison Procedures Tukey´s test, $p < 0.05$).

Chapter 6

General discussion and conclusion

The physic nut tree *J. curcas* is a rich source of phytochemicals (Devappa *et al.,* 2011). The assessment of the efficacy of the seed oil and the Phorbol esters fraction showed a high efficacy on aphids. Oils appeared to induce mortality due to asphyxia by obstruction of tracheae and tracheoles (Stadler *et al.,* 2009). However, the effectiveness of these compounds was widely depending on their mode of application. When sprayed or topically applied on the aphids, the oil covered the insect cuticle, blocked the spiracle and caused the suffocation of the aphid (Taverner *et al.,* 2001).

The spray of these extracts directly on insects was highly effective than spraying the host itself before infestation. The high efficacy of the contact application compared to the ingestion effect would limit the use of these compounds as preventive bioinsecticides. The mode of action of the PEs and the seed oil made them highly effective on the insect's cuticle. Consequently, plants should be already infested with aphids to induce mortality by spray application. Otherwise, ingesting compounds already sprayed on the plant as preventive approach would induce a low killing effect.

The root extract of *J. curcas* caused in in vitro conditions a high repellent effect on aphids. The extract from secondary roots was reported to be rich with gallic, ellaguic and coumaric acids (El Diwani *et al.*, 2009). However, a slight decrease of its efficacy was observed overtime on plants. In spite of this time-limited effect, the root extract of *J. curcas* could be considered, as a basic-extracted product (only aqueous extract), a bioinsecticide that prevented plants from aphid infestation. Nevertheless, further phytochemical analysis of the root extract would help to study its composition and understand which components induced repellency.

The seed oil of *J. curcas* caused an inhibition of reproduction on the adults of *A. fabae*. The reproduction inhibitory effect of the seed oil, shown by our study, was already reported by Ratnadass *et al.* (2012). As a response to nutrient stress, during the starvation of aphids, the fat reserves content of the soma, necessary to provide lipids to the ovaries, decreased by nearly 50% and was mobilized in profit of the insect survive (Grüber *et al.*, 1988 ; Arrese *et al.,* 2010). This insecticidal property represented an efficient barrier to the populations of aphids to reproduce and colonize plants. Although the lack of perfect

adherence of the seed oil with the leaf surface, which would permit adults to survive, these aphids would not be able to establish viable growing colonies: the limited non-treated leaf areas would not help to establish viable colonies for long time. The competition for nutrients would affect the colony growth and cause its decline.

The extracts of *J. curcas* could be an alternative for the control of Lepidopteran species. The killing effect of the PEEF differed from specie to another. It was low on the diamondback moth but high on the cotton bollworm. Inspite of the weak contact toxicity of the PEEF on *H. armigera*, the ingestion of PEEF induced a high toxic impact on the 2nd instar larvae. The PEs are analogues of diacylglycerol, an activator of protein kinase C (PKC). PKCs act as regulators of many metabolic processes. As diacylglycerol has a short biological half-life in the cell, the activation of PKC is temporary. However, the activation of PKCs by PEs, is much extended, which subsequently disrupts a number of cell process and leads to the cell exhaustion (Goel *et al.,* 2007).

A major interest should also be given to investigate further the antifeedant properties of the physic nut tree on Lepidopteran species. The deterrent property of PEEF on the early instars larvae of *P. xylostella* and *H. armigera* could be another approach in terms of plant protection. The disruption of the larval feeding behavior induced by the presence of PEEF on the feeding substrate inhibited larval growth. The antifeedant effect on the larvae of *P. xylostella* could also be caused by the ingestion of aqueous leaf extract applied on the host plant. All these antifeedant extracts affected the insect´s fitness and the reproduction performance of the subsequent adults.

The physic nut tree *J. curcas* could be an interesting source of insecticidal compounds against root-feeding insects. The seed oil and the PEEF exhibited ovicidal and larvicidal effect on the cabbage root fly *D. radicum* under laboratory conditions. Essential oils create a thin layer on the surface of eggs and stop the gas exchanges. They might also cause hardness in eggs which make it difficult for larvae to hatch (Buteler *et al.*, 2011). Although PEEF decreased the pupation rate of larvae under greenhouse conditions, many pupae were collected from the soil-treated cabbage plants. The efficacy of PEEF in semi-real conditions on *D. radicum* could be enhanced by increasing the number of times of soil treatments, for instance 4 times instead of only twice during this study. The degradability of PEs-based extracts by biotic and abiotic factors would limit their longevity in soil and reduce subsequently their efficacy on root-feeding insects.

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DECLARATIONS

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form. Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

Göttingen...............................

Signature...

Name..

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorised aid.

Göttingen,..

Signature...

Name..