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Evaluation of Biological Efficiency of Neem Oil Extract(Azadirachta Indica JUSS) as Alternative to Enthomopathogenes in the Population Dynamics of Hypothenemus Hampei, Cherry Berry Beetle of Coffea SPP in Cameroon

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Abstract

A study to find out natural enemies of the coffee cherry borer beetle *Coffea spp. hypothenemus hampei (H. hampei)* (Ferrari) (Coleoptera, Scolytidae) was carried out in two ecologically different sites of Cameroon: Moungo in the Littoral region of the country and Nkolbisson around Yaounde in central region. For six months, 600 berries were randomly collected monthly at each site. The presence of *neem* oil extract (*Azadirachta indica Juss.*) fungus was observed on berries infested by the borer beetle. Rates of borer insect induced mortality to *neem* oil extract (*Azadirachta indica Juss.)* at Melong are: 78.6% in August, 67.6% in September, 42% in October, 63.2% in November, 39.7% in December. On the other hand, these rates at Nkolbisson are: 52.6% in October, 20% in November, 17.4% in December, 11.6% in January, 8.4% in February and 1,4% in March. From these observations, it was possible to carry out a research on the pathogenicity tests through its natural presence and artificial-infection trials of the borer beetle with this fungus in order to protect better the coffee grains with a biological product.

Keywords: Coffea spp; Hypothenemus hampei; insecticidal effect; Azadirachtin A.

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1. Introduction

Coffea spp. is the most important agricultural commodity in over 70 countries, accounting for over US $70 billion in annual retail value [4] . Of the more than 100 species in the genus *Coffea* [4 − 6], only two species, *Coffea arabica L.* and *Coffea canephora* Pierre ex A. Froehner are grown commercially. Small-scale farmers produce 70% of the world's coffee, with over 100 million people depending on its production [9]. Coffee is attacked by more than 850 species of insects [1 − 2]. Of these, the coffee berry borer, *Hypothenemus hampei* (Ferrari) (*Coleoptera: Curculionidae*), is the only species that directly attacks the seed [4], and is the main threat to coffee production [3, 5], with annual losses Procceedings of the Third International Symposium on Biological Control of Arthropods, Christchurch. Exceeding US $500 million [8 − 9]. This pest was first described in 1867 in France feeding on coffee beans [8 − 10] and has subsequently spread to all coffee producing countries except Hawaii [9] and Nepal. In this paper, we review the biology and ecology of *H. hampei*, examine various biological control efforts, and demonstrate the potential for DNA-based detection

methods in unraveling trophic interactions between these cryptic pests and their natural enemies.

1.1. Biology and ecology of hypothenemus hampei

Developing coffee berries are typically attacked by single mated female *H. hampei* from between eight weeks after flowering until harvest (>32 weeks) [8]. It takes up to eight hours for adult female H. hampei to bore through a coffee berry to reach the endosperm (Fig. 1). A female *H. hampei* lays 200-300 eggs over a period of 60 days [11]. At 27 degree wwC, the egg stage averages 4.3 days, the three larval stages average a total of 12.0 days, and the pupal stage 5.2 days[12]. Due to 60 days of oviposition period, all life stages co-occur in the berry. Mating occurs between siblings within the berry [8], after which the female either remains in the berry to commence oviposition, or exits in search of another berry to colonize [11]. High temperature and relative humidity triggers emergence of mated females [4] but males spend their entire life cycle inside the berry [12]. During the interharvest period, a single berry may contain as many as 150 adult *H. hampei*, and reproduction only ceases once all resources have been consumed [16]. *Hypothenemus hampei* causes three types of damage. First, because they feed on the endosperm, there are both losses in overall yield and in quality of the beans [14]. Secondly, mature berries become vulnerable to further insect attack and infection by fungi induced the physical damage caused by the boring and feeding activities of *H. hampei* [6 − 8]. Thirdly, *H. hampei* infestations can result in arrested development, decay or premature fall of the berry [12].

1.2. Biological control of hypothenemus hampei

Species of parasitic and predatory H*ymenoptera* and *entomopathogenic* nematodes and fungi, have all been examined to various degrees for their potential role as biological control agents of *H. hampei*. The prodigious rise of the chemical industry in the twentieth century has profoundly and irreversibly changed production and consumption patterns in technologically and economically advanced regions as well as in less wealthy regions of the world. In particular, massive production and the widespread use of chemicals in agriculture, particularly mineral fertilizers and plant protection products, have made it possible to intensify agriculture with a dramatic increase in crop yields. The environmental awareness of the issue of the harmfulness of chemical substances that has gradually developed during the last decades of the last century now poses with some seriousness the question of chemical safety which requires an integrated approach to pest management combining different techniques and methods as in the field of pest management of crop and disease vectors. At the same time, concern for the quality control of various environmental compartments in relation to their level of contamination by toxic chemicals such as pesticides, fertilizers and other pollutants has become a major international concern especially since the Rio Conference in 1992 on development and the environment. Inscribing ourselves in these international concerns and having in view their implications in the specific context of Cameroon, it seemed to us pertinent to ask ourselves about the environmental balance sheet; in terms of assessing the risks of exposure of populations to persistent organic pollutant pesticides as a search for alternatives to control the coffee berry borer, we evaluated in the laboratory the insecticidal effects and of the seed oil of *neem* on the coffee berry borer *H. hampei* (Ferrari, 1867).

Figure 1: Damage caused by *Hypothenemus hampei*.

1.2.1 Chemical fight

Chemical control consists to use the chemicals (insecticides, pesticides). In the early 1970 and like other countries in world, Cameroon prohibits the use of persistent organic pollutants pesticides one after the other. Organochlorine pesticides have been gradually replaced by *organophosphorus* and synthetic *pyrethroids.*

However, the appearance of the resistance of *H. hampei* synthetic *pyrethroids* necessitated the release of new treatment programs including endosulfan and insecticides such as organophosphates.

1.2.2 Cultural, agronomic or mechanical control

It groups together all the measures creating unfavorable conditions for the development of the pest. Sanitary harvesting which consists to pick up the inter-country cherries left on the branches or fallen on the ground and to destroy them is one of the main ones. The cultural practices such as the size of the coffee trees, the regular weeding and the cultivation of the same variety allowing a maturation are appropriate measures to reduce the risk of attacks [5]. The debarking of fallen logs makes it possible to avoid that bark beetles settle under the bark to lay eggs. Adults are attracted by the smell of trees, especially sick ones or in physiological deficiency in case of drought. This phenomenon makes it possible to attract them to odorous traps reproducing the odor spectrum of diseased trees [6].

1.2.3 The biological struggle

Biological control of coffee berry borer consists in using its own natural enemies to limit its population. The main ones are the parasitoids *C. stephanoderis* and *P. coffea (Hymenoptera*) and the *entomopathogenic Beauveria bassiana*, a cosmopolitan mushroom that attacks more than 200 species of insects. These natural enemies of the bark beetle are often used as a means of control to control its population [9].

1.2.4 Integrated pest management

Integrated pest management combines all possible and useful control methods against the pest. It includes trapping, the best planting product, biological control and rational use of pesticides.

1.2.5 Parasitoids: The fight by using neem oil extract (Azadirachta indica A. Juss)

The *neem (Azadirachta indica A. Juss)* also caught our attention for its uses in traditional agriculture. Observations from local know-how show that farmers in the north of Cameroon, south of the Sahara, use the leaves and seed *neem* oil extract to protect crops and attics.

1.2.5.1 The neem (Azadirachta indica A. Juss)

The *neem (Azadirachta indica A. Juss)* (Fig.1) is a deciduous tree native to the southern Himalayas. It belongs to the family Meliaceae and grows in the subtropical countries of Asia and Africa. The *neem*, called the wonderland tree or pharmacy tree of the village, is used in India and Africa for its medicinal properties and its repellent or lethal effects on pests [12]. The plant reproduces mainly by seed. The tree of the neem reaches 5 to 25 m of height according to the regions. The cracked bark is a dark brown gray with a brown red slice. The leaves are imparipinnate alternating 20-40 cm, lanceolate dissymmetrical at base, scalloped toothed, glabrous and dark green in color. White, yellowish or creamy flowers, arranged in axillary panicles. The fruit is an ellipsoid drupe of yellow-green color, with a thin epidermis and a juicy pulp when ripe. Infused in water, the leaves of *neem* are a traditional remedy for malaria in Africa. Fruit oil is a natural product whose extracts have an extremely toxic and non-mutagenic effect on insects, but remain harmless to humans and warm-blooded animals [8]. The protection of dry legumes with neem oil has been very successful in northern Cameroon, Benin and Niger [13].

Figure 2: Plant and fruits of *neem (Azadirachta indica A. Juss)*.

1.2.5.2 The action of neem oil extract on the bark beetle (H. hampei)

A new approach of control deviates imperative all the more as this insecticide is already classified internationally as a persistent organic substance. With a view to moving towards more ecological alternatives capable of replacing including endosulfan in coffee growing, we chose as the target insect coffee berry borer (*H. hampei)*. The objective of the study is to evaluate the insecticidal potential extracts of some local plants (*neem* seed oil)) on the bark beetle. Since the control alternative sought here is intended above all to respect the environment and can constitute a component of an integrated control approach against bark beetle, it is interesting to know the effect of *neem* oil extract on its main parasitoids *Cephalonomia stephanoderis* [12 − 15] (*Hymenoptera: Bethylidae*) and *Phymastichus coffea* (*Hymenoptera: Eulophidae*).

1.2.5.3 Experimental framework and equipment used

The biological tests were carried out in the entomology laboratory of the Agricultural Research Institute, Forest Zone at Nkolbisson in the coffee producing area. The laboratory has a breeding room equipped with a thermometer and a humidifier. The natural populations of *H. hampei, C. stephanoderis* and *P. coffea* constituted the biological material. The *neem* oil extract, whose physical and chemical characteristics are presented in the appendix, and the commercial reference insecticide, endosulfan (EC 500 g.L^{-1}), are the active ingredients used. The small laboratory equipment consisted of breeding boxes, emergence crates, the mouth aspirator (Fig.2), specimens and beakers, insect probing needle, magnifying glass and micropipette. Gilson brand, glass bottles, mosquito net and stopwatch.

1.2.5.4 Preparation of the seed oil of neem

The Seeds of *neem* ripe and dried at 40degree C in an oven for three days were dehulled and cured. The collected almonds are ground using a coffee grinder. The paste obtained is kneaded by hand with a few drops of water to extract the oil. Seeds of *neem* ripe and dried at 40 degree C in an oven for three days were dehulled and cured. The collected almonds are ground using a coffee grinder. The paste obtained is kneaded by hand with a few drops of water to extract the oil.

1.2.5.5 Insect collection

The larval stages of the three insect species occur within the coffee cherries and only the adult stages move and are readily available. For this reason, we chose the adult stage of the three insect species. The cherries ”*scolytes*” inter-campaigns are first harvested in farmers’ plantations and on the stations of the Institute for Agricultural Research in forest area. They are then placed in breeding boxes and emergence crates in the laboratory (Fig.3). Finally, using a vacuum cleaner mouth, emerging adult insects are recovered as and when biological tests.

Figure 3: Material used in biological tests: 1 breeding box containing cherries, 2 Emergence Box and 3 Three Mouth Vacuums for Recovery of Emerging Insects.

1.2.5.6 Dose preparation of active ingredients

The concentrations were determined according to a geometric progression of reason r= 1.7782. After several preliminary tests, 1000g.mL^{-1} was selected. It is prepared by diluting seed *neem* oil extract in distilled water. Two droplets of emulsifiable surfactant (native soap) are added in the dilution to achieve a stable emulsion. The susceptibility of the bark beetle (*H.hampei*) to *neem* oil extract is higher than that the parasitoid *C. stephanoderis*. This denotes the selectivity of natural plant extracts that happily kill the pest more than its natural enemy. The *neem oil extract* seems the most selective. These observations overlap with those found in the literature [12] indicating that neem does not appear to be toxic for some parasitoids belonging to the order Hymenoptera. The parameters usually studied in the effect evaluation tests of *neem* oil extract on parasitoids are: level of parasitism, adult survival (mortality), development, passage from larval stage to adult stage, life span and palatability. Simmonds and al. [10], tested two formulations of *neem* (a crude ethanol extract containing 1% *azadirachtin* and *Azatin* EC, 3% *azadirachtin*) on the parasitoid *Encarsia Formosa* and its host, the whitefly or whitefly (*Trialeurodes vaporarium, Hemiptera*) (a parasite of tomato and green beans). They concluded that they did not observe a toxic effect of two formulations of the *neem* oil extract on *Encarsia Formosa* adults. formosa. Lyon and al. [13] demonstrated that products containing azadirachtin at functional concentrations have minimal effect on *Trichogramma minutum* when used for the control of *Ephestia kuehniella* (cereal grain meal parasite).

1.2.5.7 Compared product-dose effect on **Hypothenemus hampei** *and andosulfan pesticide (EC 500 g.L^{-1})*

Table 1: effect of different doses of andosulfan and aqueous extract of neem seeds on the pest H.hampei.

treatment	***Repetion in august*** (%)	***Repetion in september*** (%)	***Repetion in october*** (%)	***Repetion in november*** (%)	***Repetion in december*** (%)	***Average*** (%)
Untreated control	***66,00***	***76,00***	***68,00***	***56,00***	***66,00***	***66,40***
Reference product andosulfan	***63,33***	***57,73***	***64,01***	***62,47***	***52,73***	***60,04***
***Extract of neem oil* 1L/ha**	***64,24***	***65,51***	***66,83***	***69,37***	***64,66***	***66,11***
***Extract of neem oil* 1,5L/ha**	***78,00***	***77,11***	***73,54***	***78,99***	***75,49***	***77,39***
***Extract of neem oil* 2L/ha**	***84,19***	***81,25***	***81,06***	***82,75***	***80,28***	***81,90***

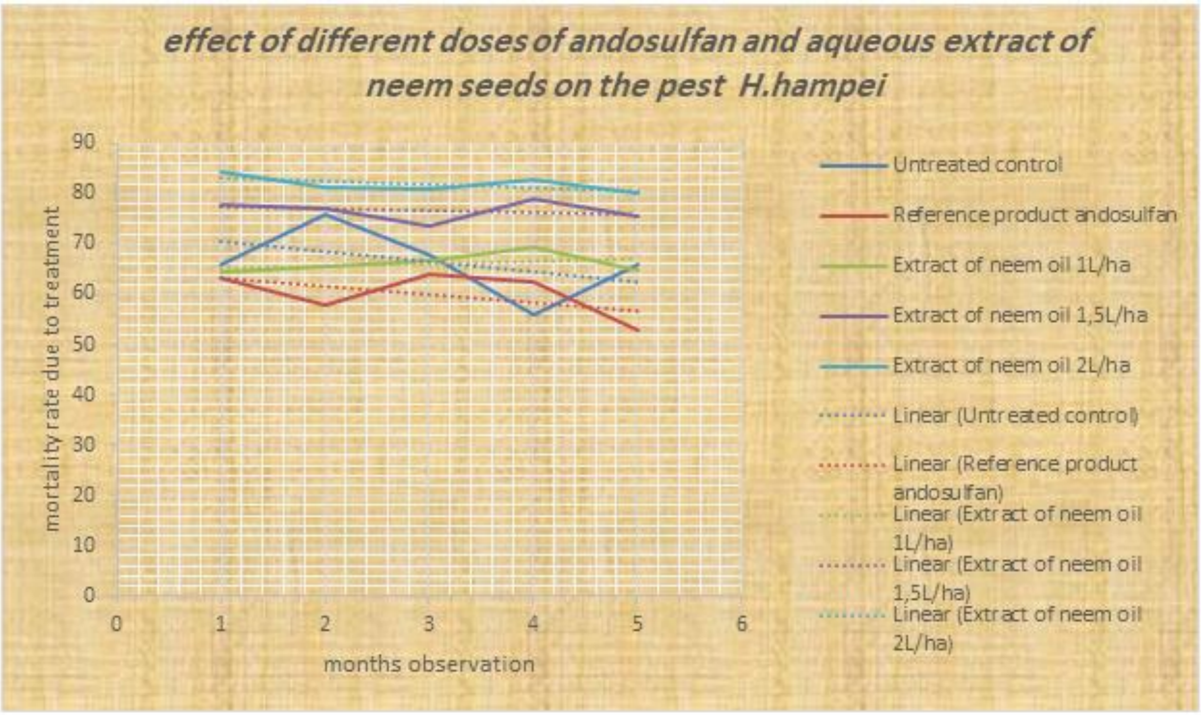

Figure 4

The results show a significant positive effect of *neem* oil extract to reducing attacks by *H.hampei* bark beetles on coffee trees. These results appear as further proof the proprieties of the main compound with insecticidal proprieties *neem* already mentioned by a number of researchers. In general, the *neem* oil extract tested affects the pest *Hypothenemus hampei* more than its the andosulfan pesticide (EC 500 g.L^{-1}). As a result, it may be compatible with the integrated management program for bark beetle populations, application of insecticides based on *neem oil extract* in combination with biological control in coffee plantations.

2. Conclusion

This experiment carried out in the open field has once again clearly confirmed the effectiveness of the *neem oil extract*. This substance applied at a dose of 1/ha is statistically as effective as the product of reference to its approved dose andosulfan. She is able to significantly reduce the incidence of coffee berry drop. *The neem oil extract* also have properties systematically against leafminers and forest defoliators when they are inoculated into the trunk of trees. Acting against the dynamics of the coffee berry borer, its insecticide property could not constitute a doubt and can play a role Important in the integrated fight in our cultures and even constitute an alternative to the pesticides of synthesis whose use is not without effect on man and his environment.

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