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Invitro Nematodicidal Effect of the Aqueous Extract of *Nicotina tabacum* (Solanaceae) Leaves on *Ascaridia galli*, A Parasite of the Small Intestine of Chickens

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A B S T R A C T

Nicotina tabacum is a plant native to South America of the Solanaceae family and is widely used in the African pharmacopoeia to combat several diseases, including digestive parasitosis. The objective of the present study was to investigate the in vitro nematodicidal activity of the aqueous extract of N. tabacum leaves against Ascaridia galli parasites of the small intestine of chickens. The study consisted of a contact mortality test. Harvested A. galli adults were exposed to seven drug preparations including six different concentrations (4, 8, 16, 32, 64, and 128mg/ml Phosphate Buffer Saline) of the aqueous plant extract and a positive reference preparation (Levamisole at 4mg/ml PBS). The PBS served as a negative control. The test was repeated six times. The results obtained showed, after 8 hours of exposure, significant mortalities of 50 to 100%. The study showed a dose-dependent effect during the test. The mortality rates noted could be attributed to chemical compounds (alkaloids, quinine derivatives, polyphenols, gall tannins, flavonoids, steroids and triterpenoids, saponosides, reducing compounds and coumarins) with antiparasitic effect, detected through phytochemical screening. This study shows that *N. tabacum* has a nematodicidal effect on *A.galli*. However, further tests are envisaged to confirm this efficacy.

Introduction

Livestock is one of the main activities undertaken by man to address food security issues. It represents 40% of the value of world agricultural production Agridape (2010) and contributes to the world economy in general and to that of African countries in particular. For many smallholder farmers, this sector is an important safety net and a source of meeting the protein needs of the population (Akiyo, 2008). In recent times in West Africa, the decision-making powers have given pride of place to the exploitation of short-cycle animal species, including poultry as a priority (Amoussou, 2007). Despite this contribution of the sub-sector to the economy countries, it is confronted with enormous diseases including parasitosis. Although these enemies of production do not often cause direct mortality, they

nevertheless generate declines in production and productivity (Yousfi, 2012). The anthelminthic control carried out for years with conventional molecules ended up showing its limits both on the efficiency plan (failures resulting from the phenomenon of resistance) than on the ecological plan (Houngnimassoun *et al.*, 2017). Considering the importance of the veterinary pharmacopoeia in the traditional farms in Africa and particularly in Benin, it seems obvious to promote endogenous methods of treatment or prevention of diseases. It is in this context that the present study falls, the objective of which is to evaluate the nematodicidal effect of the aqueous extract of *Nicotina tabacum* leaves on *Ascaridia galli*, a parasite of the small intestine of chickens.

Materials and methods

Plant and Animal material

The biological material consists of *N.tabacum* leaves and adult worms of the *Ascaridia galli* species collected from the small intestine of traditional chickens. The plant has been identified and certified at the National Herbarium of Benin located at the University of Abomey-Calavi. The laboratory equipment consists of an electronic load cell, a stirrer, an oven, magnifying glasses, beakers, Petri dishes, a battery of fine-mesh sieves (250 µm and 500 µm), scissors, pliers, labeled bottles, a brush, thread, a water jet system and various products (distilled water, Lactophenol, Phosphate Buffer Saline).

Harvesting and preparation of the plant extract

The leaves of *N.tabacum* were harvested mature in natural environments in Ouarou in the town of Pèrèrè in northern Benin. These leaves were washed with plenty of water and dried away from dust and sunlight for three weeks before being reduced to a fine powder using a grinder. The extraction was carried out by maceration of the vegetable powder in distilled water at the rate of 50 g of powder for 500 ml of distilled water. The mixture is placed under mechanical stirring for 72 hours then filtered with cotton and filter paper. The filtrate was put in an oven at 43°C for evaporation. The dry extract thus obtained was weighed and then stored in the refrigerator until use.

Phytochemical screening of *N.tabacum* leaf powder

The different metabolites present in *N.tabacum* leaf powder were determined by phytochemical screening at URMAPha of the University of Abomey-Calavi according to the method of (Houghton and Raman, 1998) (Table. I).

Contact mortality test on adults of *A. galli*

The method used to assess contact mortality is that described by Houngnimassoun *et al.*, (2020). This method consists in taking adult worms of *A. gallipar* helminthological autopsies after harvesting the viscera of traditional chickens freshly slaughtered on the slaughter area located at the Saint Michel market in Cotonou in Benin. Live worms collected at helminthological autopsy were rinsed and kept in PBS for testing. Eight petri dishes containing respectively PBS (negative control), the six concentrations of the aqueous extract (4, 8, 16, 32, 64 and 128 mg/ml of PBS) and Levamisole (positive control) at the concentration of 4mg/ml of PBS each received an adult worm of *A.galli* and this at ambient laboratory temperature (25-30°C). Six repetitions were done. Inhibition of motility of adult worms for 10 seconds under the effect of treatments was used as a criterion for anthelmintic activity. The mobility of the worms was observed at time intervals of 30 min, 1 h and every 2 h until the death of all the worms contained in the negative control PBS. At each observation, immobile worms were identified and observed for ten seconds to confirm their condition. The worms declared dead are gently removed from the well and placed in 10 ml of PBS solution in order to confirm their death. If, for 30 minutes, the worm regains its vitality, it is automatically returned to the medicinal solution. Otherwise, the worm is declared dead.

Statistical analyzes

The results of the phytochemical screening of *N. tabacum* leaf powder are noted – (absent) or + (moderately present). For the in vitro test, the mortality rates M (expressed as a percentage) of the worms were calculated according to the formula: M (%) = 100 * (N – No) / 6 where, N and No correspond respectively to the numbers of worms died in contact with a concentration of extract and PBS buffer on the same date. Comparisons of observed mortality rates were made by the two-tailed Z test using Statistica 7.1 software (Stat Soft, Inc, 2006). The difference between the rates was assessed at the 5% threshold.

Results and Discussion

Certificate

The leaf of *N. tabacum* was identified and certified in the National Herbarium of the University of Abomey-Calavi under the number YH 638/HBN.

Phytochemical screening and extraction yield

The various analyzes carried out for the phytochemical screening made it possible to highlight nine chemical groups in the leaf powder of *N. tabacum* (table II).

Contact mortality test

Table III summarizes the results of the mortality rates induced by drug preparations and controls. We note through this table a gradual increase in mortality rates which rhymes with the increase in concentration of medicinal products. This increase in mortality reached 100% with the highest concentration of the plant extract (128mg/ml of PBS) after two hours of exposure. On this same date, we note that the positive control (levamisole) only killed 16.66% of the parasites brought into contact. A significant difference ($p<0.05$) was noted between the mortalities recorded with the plant extract and the levamisole. It was only after 4 hours of exposure that this conventional molecule caused 100% mortality of the worms brought into contact. After six hours of contact, the aqueous extract of *N. tabacum* induced mortality ranging from 16.66% to 100% respectively with the different concentrations.

It should be noted that on this date, the negative control has not yet recorded any mortality. The aqueous extract of *N. tabacuma* leaves inhibited the motility of adult *A. galli* worms compared to the negative control (PBS) with a more pronounced effect with the highest concentrations. The lowest concentration of 4mg/ml of PBS resulted in a 50% mortality of the worms placed in contact after a period of 8 hours. During the test, the inhibitory effect on the motility of adult *A. galli* worms was dose-dependent and function of the contact time with the plant extract.

The aqueous extract of leaves of *N. tabacum* showed, through this study, a nematodicidal effect resulting in the high mortality rates recorded on *A. galli*. The mortality rates (50 to 100%) obtained after 8 hours of exposure are better compared to those obtained on the same species of parasite with the aqueous extracts of *Chenopodium ambrosioides* (16.66 to 66.66%) and *Spondias mombin* (50 to 100%) after 12 hours of contact by Houngnimassoun *et al.,* (2020). This study with the aqueous extract of *N. tabacum* on *A. galli* seems to be the first although *in vitro studies* on animal parasites have flourished in recent years. Existing studies focus on *Haemonchus contortus. This is the example of the study carried out in India by* Sastya *et al.,* (2017) *in which Nicotina tabacum* extract showed an anthelmintic effect on *H. contortus*. The high mortalities noted with *N. tabacum* could be attributed to the different metabolites present in the leaf powder of this plant. Indeed, the results of the phytochemical screening of the plant reveal the presence of nine chemical compounds, namely: alkaloids, quinone derivatives, polyphenols, gallic tannins, flavonoids, steroids and triterpenoids, saponosides, reducing compounds and coumarins.

Table.1 Colorimetric reactions for phytochemical screening

Families chemicals	Reagents (compositions)	Positive results
Alkaloids	Mayer {Potassium Iodide + Mercury Chloride}	yellowish precipitate
Flavonoids	SHINODA {Ethanol 95° + HCl (N/2) + (Mg ou Zn)}	Orange, red or purple coloring
Tannins	$FeCl3_{1}$ %	Dark blue, green or black coloring
Catechic tannins	STIASNY {HCl / Formol}	Precipitated pink
Gallic tannins	$FeCl3_{1}$ %	Blue or black tint
Anthocyanins	HCl 5% + ammonia 1/2	Red color that turns purplish-blue or greenish
Saponosides	Distilled water	Foam index: positive test if IM >1cm
Reducing compounds	Distilled water + Fehling liquor (A+B)	Bright red precipitate
Steroids & terpenoids	Lieberman Bouchard {Acetic anhydride + sulfuric acid}	Purple, blue or green/wine red coloring
Quinones	BORN-TRAGER {HCl 5% + Chloroform+Ammonia}	Pink or purplish red color

Table.2 Phytochemical screening of *N.tabacum* leaf powder

Chemical groups	*Nicotine tobacco*
Alkaloids	+
Quinone derivatives	+
Polyphenols	+
Catechic tannins	-
Gallic tannins	+
Flavonoids	+
Steroids and Triterpenoids	+
Saponosides	+
Anthocyanins	-
Leuco-anthocyanins	-
Mucilages	-
Reducing compounds	+
Coumarines	+

(+): presence; (-) : absence

Table.3 Mortality rate induced by different concentrations of the aqueous extract of *N.tabacum* on *A. galli* after 10 hours of exposure

Drug preparations and controls (mg/ml of PBS)		Mortality rate as a function of exposure time (hour)						
	0,5	1	2	4	6	8	10	
T-	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	100^{a}	
4	0^{a}	0^{a}	0^{a}	0^{a}	$16,66^{a}$	50^{b}	100^{a}	
8	0^{a}	0^{a}	0^{a}	0^{a}	$33,33^{a}$	$83,33^{BC}$	100^{a}	
16	0^{a}	0^{a}	0^{a}	$33,33^{a}$	$66,66^{b}$	100^{bc}	100^{a}	
32	0^{a}	0^{a}	$33,33^{a}$	$83,33^{b}$	100^{bc}	100^{bc}	100^{a}	
64	0^{a}	0^{a}	50^{b}	100^{bc}	100^{bc}	100^{bc}	100^{a}	
128	0^{a}	0^{a}	100^{bc}	100^{bc}	100^{bc}	100^{bc}	100^{a}	
T+	0^{a}	0^{a}	$16,66^{a}$	100^{bc}	100^{bc}	100^{bc}	100^{a}	

T-: Negative reference control (PBS) T+: Positive reference control (levamisole)
The percentages on the same column assigned different letters differ significantly at the 5% level.

This strong composition of the plant compared to that of *C. ambrosioides* and *S. mombin* could justify the differences noted between the results of the two studies. Furthermore, most of the chemical groups identified in the leaf powder of *N. tabacum* have been revealed in the literature as having nematidicidal properties (Hoste *et al.*, 2006; Brunet *et al.*, 2007; Chagas *et al.*, 2008; Houngnimassoun *et al.*, 2017, 2019, 2020). In Burkina Faso, the phytochemical analysis of the plant reveals the presence of alkaloids, coumarins, saponosides, tannins and sterols and triterpenes (Kambou and Guissou, 2011). Also in the same year, Boulogne identified in the *N.tabacum* plant, alkaloids, phenolic compounds and terpenoids. The difference noted from one study to another between the phytochemical analyzes of the plant is undoubtedly due to chemotypic variability from one country to another. However, it should be noted that some compounds are common to plants throughout the studies.

This study shows that *N. tabacum* has a nematodicidal effect on *A. galli*. However, further studies should be done to confirm the noted effectiveness. Through this study, we were able to characterize the leaf powder of *N. tabacum* harvested in northern Benin. The nematodicidal effect noted with this plant is interesting given the high mortality rates recorded with the different concentrations of its aqueous extract on *A. galli* in a short time. These effects are attributable to the chemical composition of the plant. However, the experiments must be continued with other tests (*in vivo* tests on chickens, tests on other species of parasites, toxicological tests), in order to provide breeders and agro-breeders with medicinal formulations based on of this plant.

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