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Laboratory evaluation of different host plants and *Taxodium distichum* ethanolic extract on *Nezara viridula* (Hemiptera: Pentatomidae)

Rehab, M. El-Gendy

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

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Abstract:

The different host plants and ethanolic extract of *Taxodium distichum* were evaluated on mortality and some biological parameters of green sting bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) a major pest of some economic crops. The data revealed that the adult survival on okra pod, cabbage and lettuce were 52.94, 30 and 27.27% with longevity 9.56, 7.66 and 6.8 days, respectively. In addition, the insecticidal activity of ethanolic extract of *T. distichum*, it had a more potent on controlling *N. viridula* where LC_{50} and LC_{90} were 7.49 and 14.41% after 72h post-treatment. The ethanolic extract induced noted a decline in the longevity of male (4.42, 3.14) and female (5.57, 2.71) at 5% and 10% conc., respectively in comparison to control 14.71 and 22.57 day for male and female. Finally, *T. distichum* ethanolic extract is evidence that it is a good efficient for green stink bug control.

Introduction

The green sting bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) is a serious economic polyphagous pest to most of the crops in Egypt due to its stylet penetrate the plant tissues causing damage to all developmental stage of the plant and so it is difficult to control. A few studies are known concerning feeding techniques of *N. viridula* , although *N. viridula* biology and ecology are extensively recorded (Huang and Toews, 2012). There was a limited attempt to improve the Menusan's green bean method in evaluating laboratory rearing of green sting bug on *Phaseolus vulgaris* (L.) as a diet (Gonzales and Ferrero, 2008 and Silva *et al*., 2011). The green sting bug reared on different seed and plant tissue combination as green snap beans, raw-shelled peanuts, immature radish fruits and immature soybean pods caused shorter to duration period from egg to adult (Panizzi and Saraiva, 1993; Noda and Kamano, 2002 and Gonzales and Ferrero, 2008). In Integrated Pest Management, the resistances of plant studies are techniques take part in insect population reduction (Souza *et al*., 2013).

The using increase of chemical insecticides or alternate one chemical product leads to more problems in controlling sting bugs (Musser *et al*. 2011) which aid in developing sting bug resistance to chemical insecticides and environment pollution (Hart and Pimentel, 2002 and Sosa-Gomez and Silva, 2010). Consequently, the alternative uses of plant extracts as natural pesticides are more remarkable because they have toxic bioactive compounds that low toxicity on mammalian.

Taxodium distichum (Cupressaceae) exhibited antioxidant, antitumor, cytotoxic, antiviral, antibacterial and antifungal activities (Ibrahim *et al*., 2006 and Kusumoto *et al*., 2010) because of its enrichment in glycosides flavonoids, monoterpenes, diterpenes, and sesquiterpenes. The insecticidal activity of *T. distichum* extracts was less evaluated.

The present work aims to estimate survival activity observed for *N. viridula* reared on the different host plant. Also, the toxic effect of *T. distichum* fruit ethanol extract on some biological parameters of *N. viridula* adult and determined its LC_{50} and LC_{90} were evaluated.

Materials and Methods

1.Insect source:

Adults of *N. viridula* were obtained from a colony reared on fresh green bean, *Phaseolus vulgaris* L. in the laboratory with fixed temperature of 27± 2 ^{o}C , 65±10% RH and12:12 (L:D) h. photoperiod at Pest Physiology Research Department, Plant Protection Research Institute. The ethanolic extracts of *Taxodium distichum* were dissolved in ethanol.

2. Extraction of *Taxodium distichum*:

The fruit of *T. distichum* collected from El-Orman garden Giza, Egypt and then dried under vacuum at 30 oc until and crashed into powder. About 500g of *T. distichum* powder was steeped at room temperature in ethanol for one week. The extract was then filtered, concentrated to dryness in a rotary evaporator at 50 oc.

3.Bioassay:

3.1. The effect of different host plants:

The adult of *N. viridula* fed on cabbage, *Brassica oleracea* (Brassicaceae); lettuce, *Lactuca sativa* (Asteraceae); castor leaves, *Ricinus communis* (Euphorbiaceae); okra leaves and pods, *Hibisicus esculentus* (Malvaceae) under laboratory conditions in glass jars. The food source was renewed daily. The survival percent and longevity of adult green sting bug were determined.

3.2. The effect of *Taxodium distichum* ethanolic extract:

Two concentrations of *T. distichum* ethanolic extract 5 and10% were prepared with distal water. 5µL of each concentration were applied on the tergites of each adult with the topical micro applicator and 5µL distal water for control then one pair of male and female was put in glass jar lined with filter paper and covered with organza and feeding with okra pods according to (Costa *et al*., 1998). Seven replicates for each treatment were used to evaluate the mortality percent and longevity of male and female adults, the number of deposited eggs per female and hatchability. All bioassays were established at 27°C± 2°C, RH of 65%±10 %, and photoperiod of 12 hours. The mortality data after 48, 72 and 96h were corrected according to Abbott's formula (1925).

4.Statistical analysis:

Differences among mean were analysed using Duncan's analysis of variance (ANOVA), the least significant difference by computer statistical software Costat® (2005). The significance test at probability value $p < 0.05$ was considered significant. The toxicity line of *T. distichum* ethanolic extract were analyzed with Biostat version 5 using Probit-analysis.

Results and discussion

1.Effect of different host plants on adult *Nezara viridula* survival:

Data in Figure (1) illustrated the effect of different host plants as cabbage, *B. oleracea*; lettuce, *L. sativa*; castor leaves, *R. communis*; okra leaves and pods, *H. esculentus* on survival percent of *N. viridula*, green sting bug. All host plants caused 100% survival on the first day after feeding except castor leaves caused 37.3%. While on a ninth day the adult survival on okra pod, cabbage and lettuce were 52.94, 30 and 27.27%, respectively. In the same trend, regarding Table (1) the longevity of adult green sting bug was 9.56, 7.66 and 6.8 days on okra pods, cabbage and lettuce host plants on the contrary, the lowest longevity of adult 2.5 days was recorded on castor leaves. There were highly significant differences in the longevity of *N. viridula* adult feeding on the various host plants.

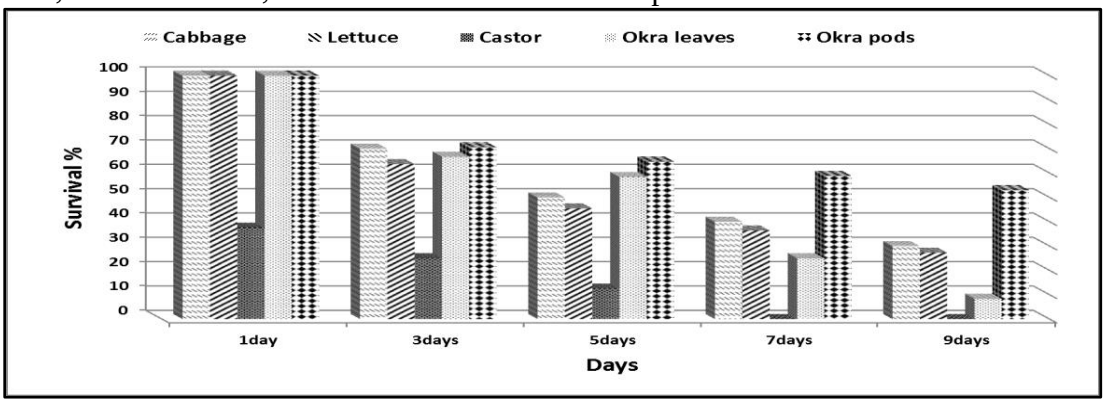

Figure (1): Survival percentage of adult *Nezara viridula* reared on different host plants.

Table (1): Effect of different host plants on *Nezara viridula* adult longevity.

Host plants	Longevity (days)
Cabbage leaves	$7.66^{b}\pm0.12$
Lettuce leaves	$6.8^{bc}\pm0.15$
Castor leaves	$2.5^{d}\pm0.28$
Okra leaves	$5.53^{c}\pm0.28$
Okra pods	$9.56^{a}\pm0.79$
LSD	1.28
P	0.0000***

L.S.D. means low significance differences at P< 0.05

2. Effect of *Taxodium distichum* ethanolic extract on mortality of *Nezara viridula* adult:

The efficacy of two concentrations (5 and 10%) *T. distichum* ethanolic extract were observed in Table (2). The mortality percent of adult green sting bug at 5% concentration was slightly decreased after 48h (7.14) then increased after 96h reached to 50%. On the other hand, the second concentration 10% exhibited more toxic after 48, 72 and 96h

and the mortality % were 35.71, 71.43 and 100%, respectively on comparison to control. The mortality of green sting bug adults increased with increasing concentrations of *T. distichum* as well as experimental duration.

Table (2): Mortality % of *Nezara viridula* adult infected with ethanolic extracts of *Taxodium distichum* under laboratory conditions.

Treatment	Conc. (%)	Mortality%		
		48h	72h	96h
Taxodium distichum	5%	7.14	21.42	50
	10%	35.71	71.43	100
Control		0.00	0.00	0.00

3.Susceptibility of *Nezara viridula* adult to *Taxodium distichum* ethanolic extract:

Regarding Table (3) and Figure (2) the lethal concentration of T. *distichum* ethanolic extract caused 50% and 90%; LC_{50} and LC_{90} mortality to green sting bug adults were 7.49 and 14.41% with slope 4.50 after 72h post-treatment. The mortality of *N. viridula* increased with increasing concentrations of *T. distichum* ethanolic extract.

Table (3): The lethal concentrations of *Taxodium distichum* ethanolic extract against *Nezara viridula.*

Ethanolic extract of	Lethal concentration (%)		Lower limit	Upper limit	slope
Taxodium distichum	LC_{50}	7.49	5.29	11.64	4.50
	LC_{90}	14.41	10.06	18.27	

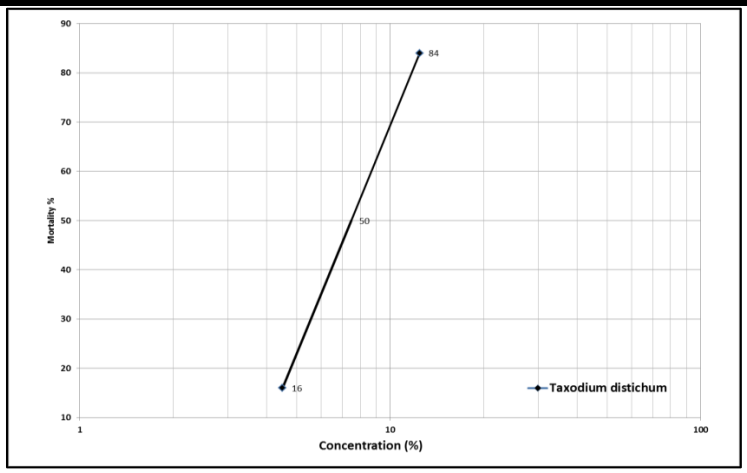

Figure (2): Probit-analysis of toxicity lines of *Taxodium distichum* ethanolic extract on *Nezara viridula* adult.

4.Effect of *Taxodium distichum* ethanolic extract concentrations on some biological parameters of *Nezara viridula* adult:

The obtained results in Table (4) and Figure (3) illustrated the longevity of male and female, mean no. of deposited egg per female and hatchability% of green sting bug. There were highly significance differences between all treatments in various observed biological parameters. The male longevity was 4.42, 3.14 and 14.71day at 5%, 10% and control, respectively. In the same context, the female longevity was 5.57, 2.71 and 22.57 days at 5%, 10% and control, respectively. There was no egg deposited at 10 % concentration subsequently, there was no hatchability. While at 5% there were 11.28 eggs with 7.49% hatchability in comparison with control was 55.71 eggs with 93.27% hatched. In conclusion, all tested biological parameters were highly decreased than the control.

Table (4): Biological parameters of *Nezara viridula* adult after treatments with *5*&10% *Taxodium distichum* ethanolic extract.

Treatments	Longevity(days) Mean+SE		Mean no of deposited Egg/female Mean+SE	Hatchability% Mean +SE
	Male	Female		
5%	4.42^{b}±0.29	5.57^{b}±0.61	11.28^{b}±4.13	7.49^{b}±3.08
10%	3.14^{b}±0.34	2.71^{c}± 0.28	0.00^{c}±0.00	0.00^{c}±0.00
Control	14.71^{a}±0.91	22.57^{a}±0.84	55.71^{a}±2.73	93.27^{a}±1.39
LSD	1.75	1.85	8.50	5.8
P	0.0000***	0.0000***	0.0000***	0.0000***

Same letters mean non-significant effect
Different letters mean significant effect at p<0.05.

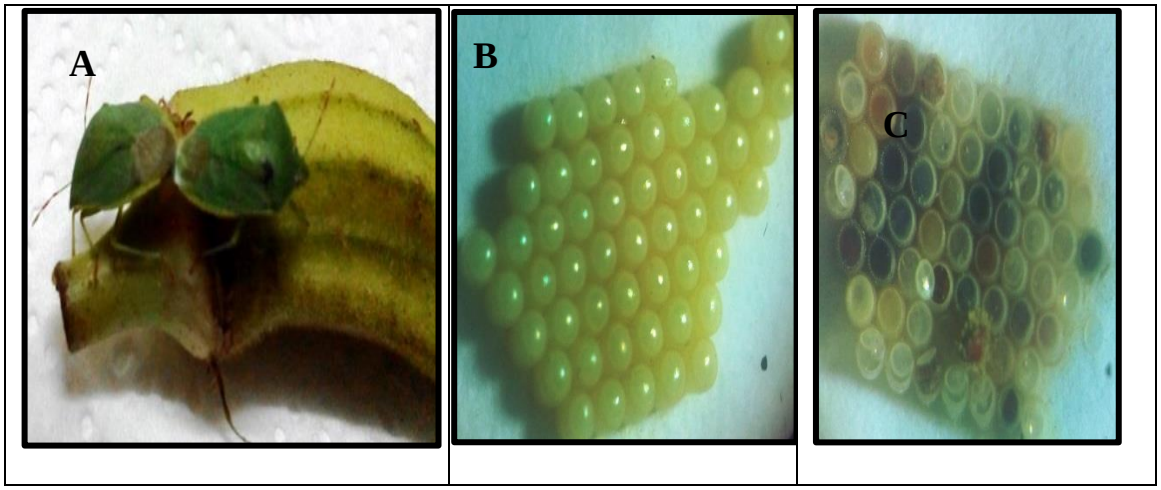

Figure (3): Effect of ethanolic extract of *Taxodium distichum* on egg deposit.

A. Mating of male and female after treatment.
B. Normal deposit egg in control.
C. Unviable egg in treatment with 5% conc.

The different host plants under investigation are effects on survival percent of *N. viridula*, green sting bug. The survivorship of adult on okra pod, cabbage and lettuce were 52.94, 30 and 27.27% and the longevity of adult green sting bug were 9.56, 7.66 and 6.8 days, respectively on contrary the castor leaves not a preferred host plant to of adult 2.5 days was recorded on castor leaves. There were highly significant differences in the longevity of *N. viridula* adult feeding on various host plants. These results are in harmony with (Panizzi *et al.,* 2000) stated that the low nymphal mortality of green sting bug reared on the artificial diet and soybean pods was 30%. In the same trend, the adult of green sting bug survival was 97.3 and 74.67% on fresh and dry yolk chicken egg-based diet, respectively (Portilla *et al*., 2015). Also, the duration of *N. viridula* reared on immature soybean pods was 90.6 d (Gonzales and Ferrero, 2008).

The concern of rearing techniques of green sting bug on different host plants is not only to investigate the population dynamics in the laboratory but also to design its control strategies. The obtained results indicated that the ethanolic extract

of *T. distichum* has insecticidal activity on *N. viridula* adult where LC_{50} and LC_{90} were 7.49& 14.41% with slope 4.50 after 72h post-treatment. These results are in the same context as the results of (Sabry, 2018) revealed that the LC_{50} and LC_{90} were 10490 ppm and 27890 ppm with slope 3.007 on 4^{th} instar larvae of *Spodoptera littoralis* after 72 hours of treatment. The contact and the stomach poison activity of petroleum ether and acetone extracts of leaves from bald cypress (*T. distichum*) were tested on adults *Tribolium castaneum* Herbst, mortality percentage increased with concentration and exposure time increasing (Shoukry *et al*., 2017).

The ethanolic extract of *T. distichum* fruits caused highly decreased in all tested biological parameters; longevity, deposited egg/female and hatchability than the control, there was no egg deposited at 10 % concentration while at 5% there was 11.28 egg with 7.49% hatchability in comparison with control was 55.71 egg with 93.27% hatched. These results are supported by Piton *et al*. (2014) evaluate the contact toxicity of the leaves acetonic extract of *Piper aduncum* (L.) on brown stink bug, *Euschistus heros* developmental stages, all concentrations tested reduced significantly the survival and reproduction in the adult bioassay. In the effect on egg contact bioassay the 8% concentration caused 19% mortality. In the same frame of reference Carneiro *et al*. (2013) found that ovicidal action of *Annonaceae* extract on the chorion of *Rhodnius neglectus* (Lent.) (Hemiptera: *Reduviidae*) eggs caused unviability of 90% of the eggs.

The mortality, low longevity and fertility of *N. viridula* adults may be related to the presence of the bioactive compounds in *T. distichum* ethanolic extract which responsible for its toxicity. (Sabry, 2018) recorded that Ferruginol, (Di-(2-ethylhexyl) phthalate), piperine, 3alpha, Didecyl phthalate and octadecane, 1-[2-(hexadecyloxy) ethoxy] are the major active compounds. Furthermore, the existence of taxodione compound; a quinone methide diterpene that possesses insecticidal activity and DNA-binding effects (Fraga *et al*., 2005 and Zaghloul *et al*., 2008). Besides, it acts as cholinesterase inhibitor (Kuźma *et al*., 2016 and Liu *et al*., 2014) and cytotoxic activities, causing apoptosis in several tumor cell lines as well as Phthalate compound is a more efficient as larvicidal activity Khatiwora *et al.* (2013).

It is concluded that the okra pod plant can be used in rearing techniques of green sting bug under laboratory conditions. The castor leaves not a preferred host plant to *N. viridula* so it can be cultivated surrounding the economic crop. The insecticidal activity and the latent effect of the biological parameter of *N. viridula* adult may be regarding the active chemical compounds of *T. distichum* ethanolic extract. These promising results are encouraged to schedule *T. distichum ethanolic* extract into insect management programs.

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