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Reinforce the Silkworms' Nutrition Using Fruits Essential oil of *Taxodium distichum*: A Prospective Way to Improve Silk Production

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ABSTRACT

Silkworms, being an important source of silk fiber, require adequate nutrition for optimal growth and cocoon formation. However, conventional methods often fall short in meeting their dietary requirements. The present study aimed to evaluate the potential of *Taxodium distichum* essential oil as a natural product to enhance vitality and silk production in the 5th larval instar of *Bombyx mori*, commonly known as mulberry silkworm. The investigation involved employing biochemical and histological approaches. Among the different concentrations tested, a 1% oil concentration exhibited the most significant effects on larval weight (5.004 g), silk gland weight (1.378 g), fresh cocoon weight (1.772 g), and cocoon shell weight (0.386 g) when compared to non-treated larvae. Furthermore, this concentration also resulted in notable improvements in several silk-related parameters such as cocoon silk ratio (21.89%), length of the silk thread (1272.96 m), weight of filament silk (0.390 g), and size of the silk filament measured at 2.212 denier (dn) when compared to other used concentrations and control groups. The tested oil was identified through GC/MS analysis, which revealed that α-pinene was the major compound, representing 74.36% of the total oil composition. Small amounts of other compounds were also identified, including Cis-Thujopsene (8.39%), Sabinene (4.09%), β-Pinene (3.08%), α-Myrcene (2.19%), and Bornyl acetate (1.34%). Histological investigation of larval brains showed no noticeable abnormal pathological changes in treated larvae compared to the negative control group. Observations revealed that the neural lamella extended continuously around the brain in a regular manner, with a dense layer consisting of neurons and glial cells beneath it. The essential oil from *T. distichum* induced significant biochemical changes in treated larvae, leading to decreased activity of acetylcholinesterase and increased activity levels of Glutathione S-transferase, Superoxide dismutase, Catalase, and Malondialdehyde when compared to their respective negative control group. In conclusion, treatment with *T.distichum* essential oil enhanced larval vitality in mulberry silkworms (*Bombyx mori*), preserved their brain structure, and improved silk production by promoting an antioxidant defense system against oxidative stress

Keywords: AChE enzyme; Brain histology; GST; MDA; Silkworm; *Taxodium distichum*.

INTRODUCTION

In Sericulture and manufacturing of natural silk; silkworm which is a monophagous insect depends on the abundance of mulberry leaves as feed meals. Thereby nutrition became the main direct factor that enhance larval growth, development, health, and capacity of silk conversion further, to raise the quality and increase the production of silk cocoons (Laskar and Datta, 2000; Waktole and Bhaskar, 2015). Recently scientists relied on food additives for mulberry leaf, to enhance nutritional contents such as vitamins, minerals, antibiotics, and hormones (Sallam *et al*., 2018; Yamani *et al*., 2020) Consequently, the addition of botanical extracts with mulberry leaves as supplement were focused in many studies to improve the nutritional value of silkworms food. (Hipparagi *et al*., 2001; Murugesh and Mahalingam, 2005). Basically, plants are the most potential insect-behavioral modulatory agent due to their bioactive compound constitutes (Kim *et al*., 2002; Rao *et al*., 2005) which have different modes of action act on repelling, attracting, feeding or feed-deterring insects (Koul *et al*., 2008). They play the main role in plant insect interactions. Terpenes are the most dominant constituent in plant volatile oils (Isman, 2007). They were described as potential insecticides; which affecting most insect systems especially the nervous system, through acetylcholine-esterase inhibition or blocking the octopamine receptors. (Sendi, and Ebadollahi, 2014). Essential oils may affect octopamine receptors and modulate insect neurotrans-mitters, neurohormones, circulating neurohormones, and neuromodulators. Relating studies observed that eugenol simulates the role of octopamine and elevates intracellular calcium level in cultured cells of *Periplaneta americana* and brains of *Drosophila melanogaster* (Enan, 2005).

The terpenoids that cause insect attraction are highlighted in many studies, like Katerinopoulos *et al*. (2005) when reported that 1,8-cineole extracted from Rosemary, *Rosmarinus officinalis* L. causes attraction to *Lobesia botrana* moth and *Frankliniella occidentalis* thrips. Experimentally, Sandalwood oil, basil oil, and grapefruit oil intensively attract greenhouses' whitefly (Gorski, 2004). On the other hand, terpenoids attract beneficial insects to their hosts for pollination (Harrewijn *et al*., 2002).

Considering the *T. distichum*; all aerial parts of the tree were used in traditional medicine to treat wounds, diarrhea, and bronchial diseases. In contrast, the essential oils of Taxodium leaves and fruits were used in folk medicine to treat inflammation, infections, and various

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skin, gastrointestinal, and respiratory ailments (Al-Sayed, 2018). Besides ornamental use, the heartwood is used for building materials and has biocidal activity against the attacks of subterranean termites (Su *et al*., 2013) the chemical composition of *T. distichum* essential oil from the leaves and fruits growing in Italy and Nigeria was determined by Flamini *et al*. (2000) and Ogunwande *et al*. (2007). While El Tantawy *et al*., 1999 identified the essential oil components obtained from the fruits of *T. distichum* growing in Egypt. The essential oil was compromised monoterpene hydrocarbons, mainly α-pinene, myrcene, β-pinene, limonene, camphene and α-terpineol and α-pinene was present in the amount of 81.9% up to 94.3% during all seasons Djapic, (2022). The current study exhibits the prospective benefits of enhancing mulberry leaves with non-nutritional materials; the essential oil of *T. distichum* as attractant or fed modulator and antioxidant to enhance health and vitality of *B. mori* larvae and improved the sericulture.

MATERIALS AND METHODS

Plant collection and oil extraction

Approximately two kilograms of *T. distichum* fruits were collected from Orman Botanical Garden in Giza, Egypt during May 2019. The fresh fruits were thoroughly cleaned and crushed using a blender. The resulting paste was then mixed with tap water and subjected to extraction. Hydro-distillation technique was employed for extracting the volatile oil from the fruit paste in a clevenger type apparatus, with each batch consisting of 500 grams of paste extracted for a duration of five hours. The obtained oil exhibited a deep yellow color along with a strong aromatic odor. Excess water present in the oil was removed through the use of anhydrous sodium sulfate, following which it was refrigerated until further use (Khater and El-Shafiey, 2015).

Silkworm culture and rearing techniques

Silkworm egg batches, Bulgarian hybrid (H1 x KK x G2 x V2) from the Sericulture Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center, Giza, Egypt were obtained. All experiments were archived in laboratories of Sericulture Research and Pest Physiology Departments of Sharqia Branch of Plant Protection Research Institute, Agriculture Research Center, Egypt.

Eggs of silkworms are incubated to hatch and kept in a rearing chamber at laboratory conditions (28 ± 2 °C , 70 ± 5% RH and 12 h. of light, , 12 h of darkness) by the method of Krishnaswami (1978). The 1st instar larvae were fed on mulberry leaves *Mours alba* var. Balady (native). The whole set of clean rearing container and the daily replacement of paper sheets that cover the bottom of container as well as removed feces and residue of dry leaves. Principally, the rearing chamber and all tools were washed and sterilized by 3% formalin.

Experimental assay

M. alba leaves were treated with gradient oil concentrations of *T. distichum* by atomizers and dried then provided to 2nd and 4th days of the fourth instar larvae of *B. mori* which divided into five groups. Each group included three replicates plate containing 100 larvae in each plate. Four groups represented the oil treatments that sprayed with the tested concentrations of 1, 0.5, 0.25, 0.13 % while the last group represented the untreated control group. All groups left to complete feeding process on untreated clean mulberry leaves till reaching cocoons. Feces and food wastes were removed daily with cleaning nets (Zannoon and Shadia 1994). When the cocoons complete their formation the larvae began to develop into pupae inside it. Random samples of about five fresh cocoons were picked from each group to investigate cocoon general characteristics such as cocoon fresh cocoon weight, shell weight and cocoon silk ratio. The latter was calculated by the method of Tanaka (1964) as follow:

$$\text{Cocoon silk ratio (\%)} = \frac{Cocoon\,shell\,weight}{Fresh\,cocoon\,weight} \text{ X } 100$$

For technological measurements, five cocoons of each concentration were dried in an oven at 60 °C for 8 h to be reeled individually. The length of reeled silk filament was measured and weighed for each cocoon. The reeled silk filament size (denier) was estimated by Tanaka (1964) formula:

$$\text{Silk filament size (den)} = [(Weight\ of\ silk\ filament\ (g)/(Length\ of\ silk\ filament\ (m))] \ X\ 9000$$

GC/MS analysis

The collected oil sample was subjected to analysis using GC/MS (Gas Chromatography/Mass Spectrometry) employing an HP 6890 Series A (Agilent) instrument. The analysis was conducted on a Thermo Scientific TR-5MS capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness), which is composed of a 5% Phenyl Polysil Phenylene Siloxane stationary phase. Helium gas was used as the carrier with a flow rate of 1.00 mL/min, while maintaining the GC oven temperature at 50°C for an initial period of 5 minutes, followed by an increase to reach a final temperature of 250°C.

The injector temperature was set at 250°C, and a volume of 1µL from the oil sample was injected into the system for analysis. The composition of the oil sample was determined by comparing and matching its fragmentation pattern specified for various masses with those previously registered by Adams in his work published in1989.

To analyze and interpret the data obtained from GC/MS analysis, specialized software known as Thermo Scientific™ Xcalibur™ software version OPTON-30965 was utilized. This computer program allowed for comp-arison and identification of specific compounds based on their mass fragmentation patterns according to established references provided by Adams (1989).

Histopathological evaluation of brain samples

Histological investigations were conducted following a standardized procedure. The head capsules were carefully dissected under microscopic observation in ringer's solution and subsequently dehydrated using alcohol. For further analysis, five brains from both the treated and untreated groups were embedded in paraffin wax, sliced into sections with a thickness of 5 µm, and mounted on

slides. These brain sections were then stained with hematoxylin-eosin to enhance cellular details. Photomicrographs of the stained brain samples were captured using an optical microscope (Novel, Model no: NLCD-120)

Biochemical assays

Hemolymph of five larvae were collected by cut a tiny wound in hind pro-leg of the 5th instar larva and lit the hemolymph dropped in Eppendorf containing 10 µg of phenylthiourea. After that, all tubes were inverted several times and centrifuged for 10min at 2000 rpm samples kept in ice all time before and during assays.

The total protein

The quantification of total protein in hemolymph was performed according to the method described by Gornall *et al*. (1949), with bovine albumin utilized as a standard. The protein content was expressed in milligrams per deciliter (mg/dL).

Acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was measured using the method described by Simpson *et al*. (1964). The assay utilized 0.5 ml of AchBr substrate solution (3 mM), 200 µl of enzyme solution, and 0.5 ml of phosphate buffer (0.067 M, pH 7).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was evaluated following the method outlined by Nishikimi *et al.* (1972). The assay assessed the enzyme's ability to inhibit the reduction of nitrobluetetrazolium dye mediated by phenazinemethosulphate (PMS).

Catalase (CAT) activity

Catalase activity was quantified using a spectrophotometric method based on the principles described by Beers and Sizer (1952). The assay involved monitoring the decomposition of hydrogen peroxide (H_2O_2) at 340 nm and 25 °C, allowing for the determination of catalase enzymatic activity.

Glutathione S-transferase (GST)

The enzymatic activity of Glutathione S-transferase (GST) was measured using a Biodiagnostic kit (CAT. NO. GT2519). The assay relies on the catalytic role of GST in facilitating the conjugation between reduced glutathione (GSH) and 1-chloro 2,4-dinitrobenzene (CDNB), utilizing the -SH group of glutathione. This reaction mechanism was initially described by Habig *et al*. (1974).

Malondialdehyde (MDA)

Malondialdehyde (MDA), a well-established marker of lipid peroxidation (LPO), was measured using the Biodiagnostic kit No. "MD 25 29" based on the method described by Ohkawa *et al*. (1979). The levels of MDA released were expressed as an index reflecting the extent of LPO.

Statistical analysis

In order to assess variance in the biological and silk technological data, statistical analysis was conducted. The mean values were examined using Duncan's test ($p \leq 0.05$) as recommended by Welham *et al*. (2014), utilizing the Costat program for analysis purposes. While, all data of biochemical assays were performed using ANOVA for differences comparing means in each concentration separately using Tukey's HSD test when $p \leq 0.05$.

RESULTS

In regard to the influence of the extracted *T. distichum* essential oil on the larval, cocoon, and silk filament parameters of silkworms, *B. mori* results were illustrated a general elevation of larval vitality represented in larval and cocoon weight. These effects were parallel to the gradient of tested concentrations. In the same trend, the silk technology parameters revealed a corresponding improvement.

Table (1) presents results highlighting that a concentration of 1% *T. distichum* essential oil yielded the most favorable outcomes in terms of larval weight (5.004 g), ; silk gland weight (1.378 g), fresh cocoon weight (1.772 g), cocoon shell weight (0.386 g), cocoon silk ratio (21 .89%), as well as measurements related to the silk filament such as length (1272 .96 m), weight (0 .390 g), and size denier (2 .212). These values surpassed those obtained from other concentrations and control groups studied. On the contrary, the lowest weight of pupal weight was recorded as (1.094 g), while the highest weight of pupal weight was recorded for concentration 0.13 % of *T. distichum* essential oil, while the control record was (1.142g). The statistical analysis showed that all the concentrations of *T. distichum* essential oil extraction used gave better results, while the best one was 1% concentration.

Out of the 24 components detected and identified through GC/MS analysis, α-pinene was found to be the predominant compound, constituting approximately 74.36% of the total oil composition. Additionally, analytical data revealed small quantities of Cis-Thujopsene (8.39%), Sabinene (4.09%), β-Pinene (3.08%), α-Myrcene (2.19%), and Bornyl acetate (1.34%). Other compounds were observed in trace amounts as indicated in Table (2).

Histopathological evaluation

Our study for the histological structure of the control brain and that treated with 1% volatile oil of *T. distichum* in 5th larval instars (Figures 1-4) showed that the brain mainly consists of neural lamella; an outer sheath that envelops the brain glial cells while the central part of the brain was occupied by an intensive mass of fibrous tissue, neuropile Fig. (1A). Treated group with 1% oil had no noticeable abnormal pathological changes compared to the normal control group Fig. (1b) in the horizontal section the neural lamella extended continuously around the brain in a regular manner, under this envelope the dense layer of glial cells and neurons were spotted Figure (2 A and B).

Observation of the brain with high magnification power in the glial cells area was presented in Figure (3 A and B) and showed spherical shape glial cells, fusiform neurons cells and clear nerve fiber tracts with complete ideal structures. High magnification in the synaptic neuropile area was cleared in Figure (4 A and B) where cells of the neuropile appeared to have extensive cytoplasm which surrounds the axons and dendrites.

Biochemical evaluation

Spraying of larval diets with essential oil significantly lowered the AChE activity than that of control ($F=19.46$, $p \leq 0.0001$). Although the lowest activity (0.1989 U/mg proteins) was found in the larvae treated with the highest

Table (1): Impact of *taxodium distichum* essential oil extraction on selected biological and technological parameters of *bombyx mori* (mulberry silkworm).

Measured Parameters	Control	Treated Mulberry silkworm				L.S.D $_{0.05}$	F Value
		Essential oil Concentration (%)					
		1	0.5	0.25	0.13		
Larval Weight (g)	5.004±0.152^{a}	4.657±0.195^{b}	4.233±0.434^{c}	4.253±0.145^{c}	4.053±0.393^{c}	0.262***	17.463
Silk gland Weight (g)	1.378±0.053^{a}	1.292±0.059^{ab}	1.208±0.128^{bc}	1.134±0.044^{cd}	1.092±0.130^{cb}	0.1200***	8.161
Pupal weight (g)	1.094±0.080^{c}	1.312±0.289^{abc}	1.412±0.302^{ab}	1.526±0.182^{a}	1.142±0.092^{c}	0.279*	3.664
Fresh cocoon weight (g)	1.772±0.335^{a}	1.432±0.0507^{b}	1.334±0.086^{b}	1.68±0.149^{a}	1.422±0.194^{b}	0.252**	4.835
Shell cocoon weight (g)	0.386±0.064^{a}	0.296±0.0152^{b}	0.288±0.028^{b}	0.314±0.036^{b}	0.288±0.054^{b}	0.0569**	4.594
Shell Ratio %	21.89±1.671^{a}	20.68±0.986^{a}	21.59±1.474^{a}	18.86±3.068^{a}	20.29±3.30^{a}	ns	1.373
Silk filament Length (m)	1272.96±87.86^{a}	1058.24±37.59^{b}	994.88±50.06^{cd}	988.96±50.13^{cd}	1100.96±50.02^{b}	76.145***	20.123
Silk filament weight (g)	0.39±0.022^{a}	0.272±0.041^{bc}	0.252±0.029^{c}	0.248±0.029^{c}	0.304±0.043^{b}	0.045***	14.73
Silk filament size (dn.)	2.212±0.152^{a}	1.858±0.324^{b}	1.826±0.229^{b}	1.803±0.165^{b}	1.99±0.353^{ab}	ns	2.202

All data are represented in mean ±SD. Statistical analysis were performed using ANOVA for comparison means the ; for each measured parameter, different letters per row are significantly different based on Duncan multiple range test ($p \leq 0.05$); ***, high significant value; **, moderate significant value; *, significant value.

Table (2): Phytochemical composition of essential oils extracted from fresh fruits of *Taxodium distichum* using gas chromatography/mass spectrometry (GC/MS) analysis.

No	Compound name	Classification	Retention time (Rt, min)	Molecular weight	Chemical formula	Area %
1	Delta-3-carene	Monoterpene	4.42	136.24	$C_{10}H_{16}$	0.47
2	ß-Thujene	Monoterpene	4.49	136.24	$C_{10}H_{16}$	0.32
3	ß-Pinene	Monoterpene	4.7	136.24	$C_{10}H_{16}$	74.36
4	Camphene	Monoterpene	5.15	136.24	$C_{10}H_{16}$	0.67
5	Sabinene	Monoterpene	5.74	136.24	$C_{10}H_{16}$	4.09
6	ß- Pinene	Monoterpene	5.91	136.24	$C_{10}H_{16}$	3.08
7	α- Myrcene	Monoterpene	6.17	136.24	$C_{10}H_{16}$	2.19
8	α-Humulene	Monomonocyclic sesquiterpene	7.09	204.4	$C_{15}H_{24}$	0.32
9	D- Limonene	Monoterpene	7.5	136.24	$C_{10}H_{16}$	1.28
10	Terpinene	Monoterpene	8.52	136.24	$C_{10}H_{16}$	0.44
11	α-Terpineolene	Monoterpene	9.5	136.24	$C_{10}H_{16}$	0.17
12	4-Terpineol	Monoterpene alcohol	13.52	154.3	$C_{10}H_{18}O$	0.6
13	Linalyl propionate	n-monoterpene	14.27	210.3	$C_{13}H_{22}O_2$	0.15
14	Bornyl acetate	Monoterpene	17.8	196.29	$C_{12}H_{20}O_2$	1.34
15	Widdrene (Thujopsene)	Sesquiterpene	22.58	204.35	$C_{15}H_{24}$	0.2
16	α- Funebrene	Sesquiterpene	23.1	204.35	$C_{15}H_{24}$	0.15
17	Trans- Caryophyllene	Sesquiterpene	23.26	204.36	$C_{15}H_{24}$	0.27
18	Muurolene	Sesquiterpene	23.48	204.35	$C_{15}H_{24}$	0.18
19	Cis-Thujopsene	Sesquiterpene	23.94	204.36	$C_{15}H_{24}$	8.39
20	α- Chamigrene	Sesquiterpene	25.85	204.36	$C_{15}H_{24}$	0.14
21	α- Longipinene	Sesquiterpene	26.76	204.36	$C_{15}H_{24}$	0.18
22	Cuparene	Sesquiterpene	27.05	202.34	$C_{15}H_{22}$	0.44
23	Caryophyllene oxide	Sesquiterpene peroxide	29.92	220.35	$C_{15}H_{24}O$	0.16
24	Cedrenol	Sesquiterpene hydroxide	31.04	220.36	$C_{15}H_{24}O$	0.42
Total						100.01

concentration of 1% while the inhibition of AChE activity was significantly reduced in other lower concentrations. Thus, the two lower concentrations 0.25, 0.13 % of essential oil caused the significantly lowest inhibition of AChE (0.254 AND 0.289 U/mg protein) respectively .also a better effect of larvae vitality (Fig 5).

The activity of GST significantly increased in the larvae fed on the diet-treated by the three tested concentrations of oil in comparison with control (F=13.944291, $p \leq 0.0004$). However, no significant difference was found among the different concentrations, although a positive increase was observed when the concentration of 1% was demonstrated the highest activity of GST (Fig 6). However, as shown in fig (6) for MDA concentration, oil treatments caused a significantly increase in MDA levels compared to control (F=47.708966, $p \leq 0.0000$), worth to mention that the concentration of oil was directly raising the MDA contents in treated larvae. That indicates a slight oxidative stress with oil intake.

Moreover, oil treatments exhibited higher superoxide dismutase (SOD) activity compared to the control larvae. The highest SOD activity was observed in larvae treated with a concentration of 1% (Fig. 6), which showed a significant difference between the control and treatment groups (F=32.16486, $p \leq 0.0000$). Similar trends were observed for catalase (CAT) activity (Fig. 6), where there was no significant difference among larvae fed on lower concentrations but a significant difference was found between those and the highest concentration of 1% (F=3.9534, $p \leq 0.0354$).

DISCUSSION

About oil composition; the analysis findings are almost concordant the earlier reports in Egypt by El Tantawy *et al.* (1999) who found that the most dominant compound in *T. distichum* volatile oil was α-pinene which represented (87.3 %) it was accompanied by a lesser amount of mycene (2.0%), β-pinene (1.7%), limonene (1.3%) and isob-

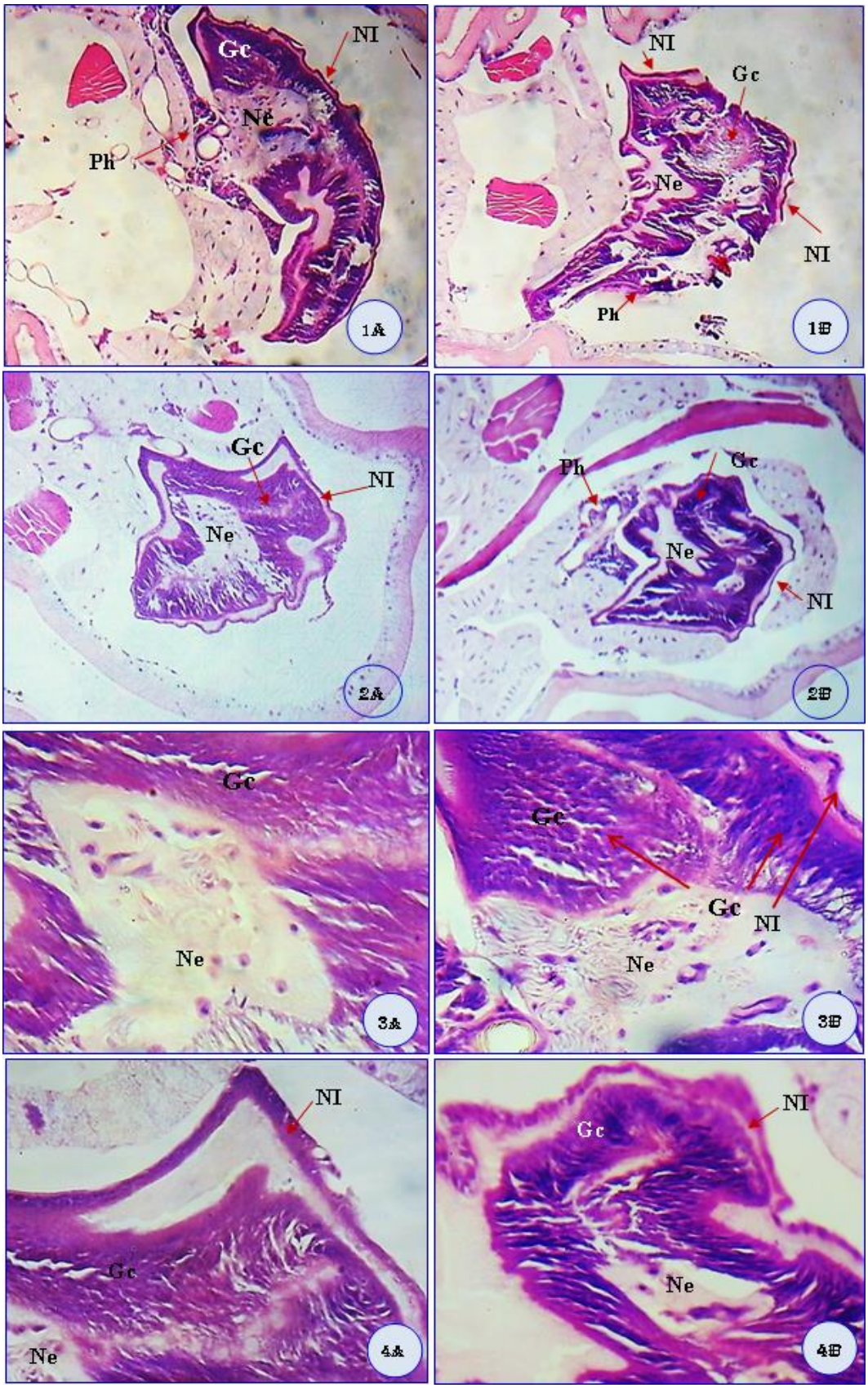

Figure (1-4): Comparison of brain structure between untreated and treated *B. mori* in 5th instar larva with 1% volatile oil of *T. distichum*. Fig. (1): Frontal section of the brain showing: A, Normal structure of untreated brain; B, Treated brain structure (200X). Fig. (2): Horizontal section of the brain showing: A, Normal structure of the brain; B, Treated brain structure displaying the outer sheath or neural lamella (NI) surrounding neurons and glial cells (Gc) area (200X). Fig.(3): High magnification image focusing on glial cells (Gc) area in the brain showing: A, Normal structure; B, Structure of treated brain, both of them have glial cells with homogenate chromatin (400 X). Fig. (4): High magnification image highlighting synaptic neuropile (Ne) area showing: A, Normal structure of untreated brain; B, Treated brain showing normal synaptic neuropils (400 X).

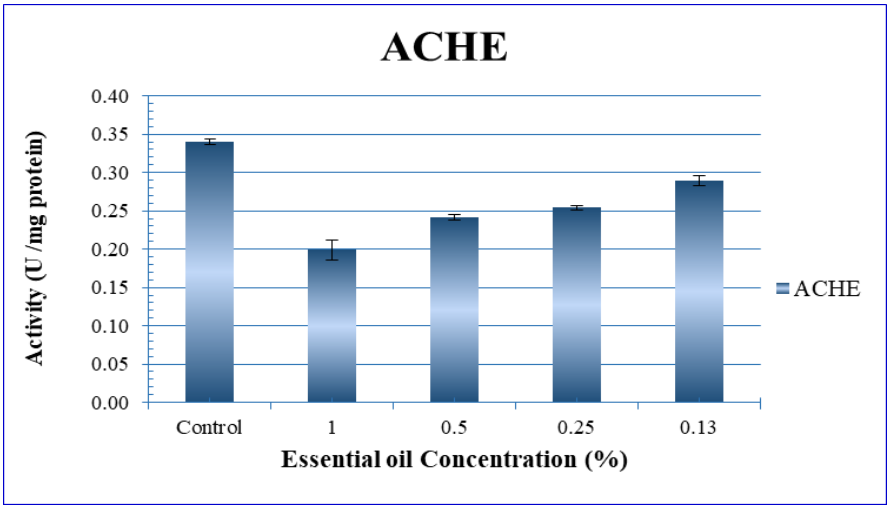

Figure (5): The enzymatic activity of acetylcholinesterase (AChE) in *Bombyx mori* larvae fed on different concentrations of *Taxodium distichum* oil. The AChE activity is expressed as units per milligram of protein (U/mg protein).

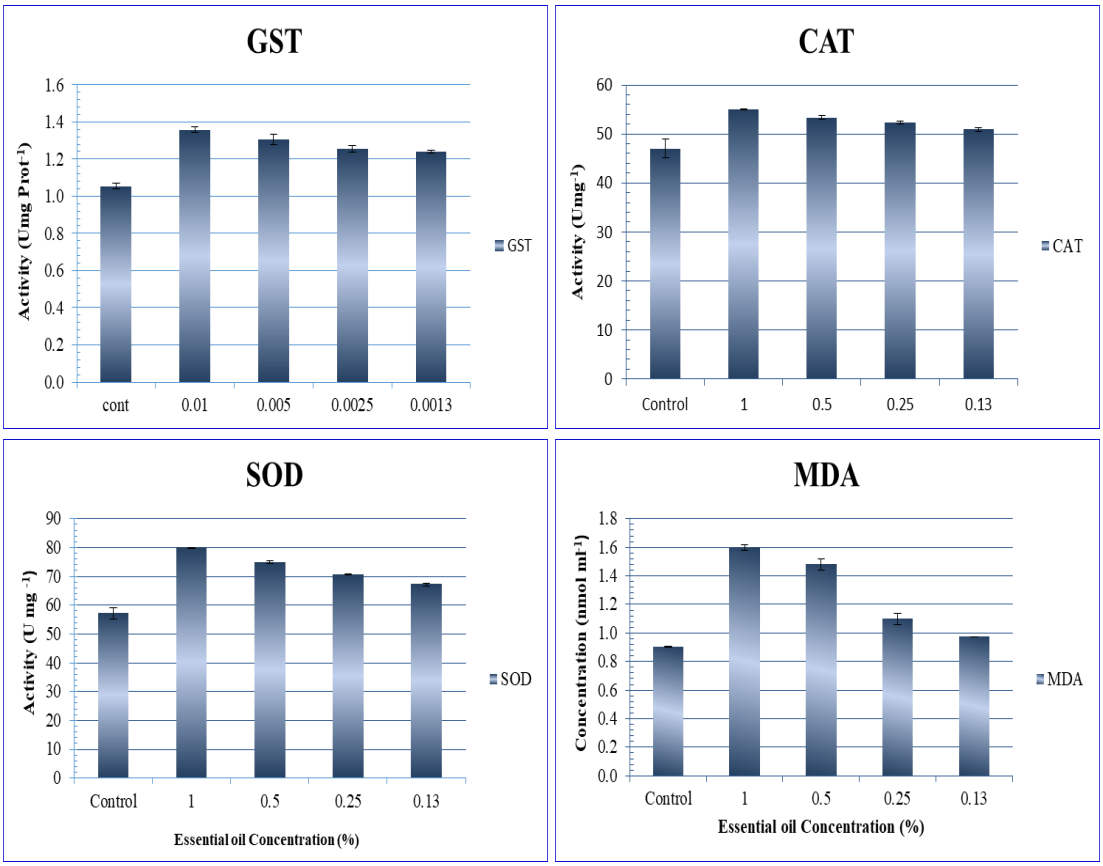

Figure (6): Responses of ROS defense system in *Bombyx mori* larvae feeding on different concentrations of *Taxodium. distichum* oil. Enzyme activity of GST, SOD and CAT expressed as U/mg protein; MDA levels expresses as nmol/ ml.

ornyl acetate (0.5%) rather bornyl acetate in cur-rent data. Also, Ogunwande *et al*. (2007) confirmed α-pinene as the main compounds in *T. distichum* fruits) 60.5%).

Constantly, the bioactivity of essential oils is directly related to their chemical composition (Tawatsin *et al.*, 2006). Thus, predictably that, α-pinene monoterpenoid which represents more than (75%) *T. distichum* oil is responsible for its bioactivity. In recent reports α-pine-ne exhibits antimicrobial and anti-inflammatory properties and acts as a broad-spectrum antibiotic also an acetyl cholinesterase modulator (Pajaro *et al*., 2017, Salehi *et al*.; 2019 and Park, *et al.*, 2021).

Histopathological examinations in this study were carried out to understand the effect of the essential oils constituents on brain structure activity, so we cannot conclude that, neither untreated group nor treated groups had abnormal pathological changes, the brain

seemed with spherical typical glial cells, fusiform neuron cells and nerve fiber tracts looked clear with complete structures.

The addition of an appropriate amount of *T. distichum* volatile oil onto mulberry leaves can enhance the brain health and reduce damage induced due to food contam-ination. Many workers came with similar results when used the same volatile oil which exhibited pronounced cytotoxic activities against PC-3, Hep G2, and Hs 578T human tumor cell lines at different investigated concentrations Ogunwande *et al.* (2007). In a similar study, Wang *et al.* (2015) demonstrated that exposure of *B. mori* larval brain to phoxim resulted in brain damage, while treatments with CeCl3 were found to reduce these damages. Conversely, Sakr and Hassab El-Nabi (2007) observed histological variations induced by *Streptomyces lavendulae* culture filtrate on the 2nd instar larvae of *Spodoptera littoralis*. They reported clear signs of degeneration and deformations in the brain tissues such as vacuoles occurring in the synaptic neuropils five days after treatment and in areas where glial cells were present.

Several reports indicate that monoterpenoids work on inhibiting acetylcholinesterase activity of insects (Houghton *et al*., 2006 and Abdelgaleil, *et al*., 2009). According to Bakkali *et al.* (2008), the mono terpenes that may be essential and lipophylic, successes penetrating cell walls and consequently pass through cytoplasmic membranes affecting membrane transport, disturb ion equilibrium and change membrane potential and quickly interfere with the physiological functions of insects.

Certainly, cumulative of Reactive oxygen species (ROS) during growth causes the organism pathogenesis and alliterating the physiological functions. All organisms can catch and restrict the reactive oxygen stress by antioxidants prior they oxidize molecules in addition to reduce the oxidized one (Steenvoorden and Henegouwen, 1997; Blokhina *et al*., 2003; Pinnell 2003). Frontline antioxidant enzymes are SOD, CAT, and GST which limit the propagation of ROS in cells (Biewenga *et al*., 1997). Glutathione S-transferase (GST) is a detoxifying enzyme that stimulates the removal of lipid peroxidation or hydroperoxides products from unhealthy cells (Huang *et al*., 2017). Malondialdehyde (MDA) is a vital component which is produced due to lipid peroxidation, ROSs induction of MDA, and production of toxic stress within cells (Wang, 2001).

In fact, essential oils directly affect insect physiological processes. One of the most important and vital processes is immune responses that induce cell death and tissue damages (Tak and Isman, 2016) this might be occurred due to generating ROS as a result of essential oil intake. So, the suggested point of our result is that the oil may regulate and promote the ROS defense system to protect cells from oxidative stress. The chosen oil gave a better cell protectiveness at the highest tested concentration of 1% while the lower concentrations; 0.5% 0.25% and 0.13% showed gradient levels of protectiveness respectively. In spite of the inhibitory action of (1% concentration) on the AChE which seems to be temporary, sometime the accumulation of acetylcholine substrate in nerves ends not accrues because the oil bind rather to the enzyme-substrate complex than to the enzyme itself and thus prevent product formation as suggested by Jankowska *et al*. (2017). This effect was reflected on the general vitality of the tested larvae and silk production where the highest concentration was more effective but not recommended to application. Further, the obtained results are consistent with each of the histopathological studies, which showed that there are no abnormal pathological changes in the untreated or treated groups, with globular glial cells, nerve fiber pathways and spindle neurons. Mostly, distinct structures were appeared.

Consequently, adding an appropriate amount of volatile *T. distichum* oil to mulberry leaves probably can enhance brain health and reduce damage from food contamination. The biochemical evaluation explains the biological and productive results obtained, as feeding larvae on rations containing essential oil led to acetylcholinesterase activity to be decreased significantly, while GST activity increased in significant level in larvae fed with the treated diet with the tested oil concentrations. No significant difference was found between the different concentrations, although a positive increase was observed when the 1% concentration was demonstrated to be the highest GST activity. Detoxifying enzymes as Glutathione S-transferase (GST) is catalyzed to remove lipid peroxidation products, or hydroperoxides, from damaged cells. The oil treatments induced a marked increase in MDA levels in contrast to the control ones, noteworthy that the oil concentration directly increased the MDA content in the treated larvae.

CONCLUSION

Based on the findings of this study, it can be concluded that the naturally derived volatile oil from *T. distichum* holds significant importance and potential in enhancing brain health in mulberry silkworms (*B. mori*). The key highlight of our results is the oil's ability to regulate and promote the ROS defense system, thereby providing cellular protection against oxidative stress. These findings underscore the potential application of *T. distichum* oil as a natural therapeutic agent for promoting brain health in silkworms and potentially other organisms as well. Further research should be conducted to explore its mechanism of action and potential applications in other contexts or species.

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تعزيز تغذية ديدان القز باستخدام الزيت العطري لثمار *Taxodium distichum* كطريقة مستقبلية لتحسين إنتاج الحرير

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الملخص العربـــــي

استهدفت الدراسة تقييم الزيت العطري لثمرة شجرة *Taxodium distichum* كعامل حيوي وتاثيره على بعض العوامل البيولوجية والتكنولوجية على العمر الخامس من دودة ورق القز *Bombyx mori* . واوضحت الدراسة ان التركيز 1٪ هو الأكثر فاعلية في زيادة أوزان اليرقات والغدة الحريرية والشرنقة الطازجة وصدفة الشرنقة إلى (5.004) و (1.378) و (1.772) و (0.386) جم على التوالي. وصلت نسبة حرير الشرنقة إلي (21.89٪) و سجلت قياسات الخيط الحريري الأكثر طولا (1272.96 م) وأعلى وزن لخيوط الحرير (0.390 جم) وحجم خيوط الحرير (2.212 دسن). بالمقارنة مع التركيزات الأخرى المختبرة والكنترول. تم تحليل الزيت المختبَر باستخدام تحليل GC / MS؛ تم تسجيل α-pinene باعتباره العنصر الرئيسي الذي يمثل (74.36٪) من الزيت الكلي بينما كانت الكميات الصغيرة من المركبات (8.39 ٪Cis- Thujopsene ، Sabinene (4.09٪ (، 3.08) β- Pinene ٪ α- Myrcene (، 2.19)٪) وخلات البورنيل (1.34٪). اوضحت الدراسات الهستولوجية عدم وجود تغيرات مرضية غير طبيعية ملحوظة فى بنية الدماغ النسيجية مقارنة بالكنترول حيث امتدت الصفيحة العصبية متصلة حول الدماغ بطريقة منتظمة ، وتحت هذا الغلاف تم الاستشهاد بطبقة كثيفة من الخلايا العصبية والخلايا الدبقية. واثبتت الدراسات البيوكميائية على أن الزيت المختبر *T. distichum* يقلل بشكل كبير من نشاط AChE ويزيد بشكل كبير من نشاط مستويات GST و MDA. بالإضافةإلى نشاطًا أعلى من SOD و CAT مقارنةً بالكنترول.