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# Nematicidal Activity of Some Chemical and Bio – Control Agents Against 2ND Stage Juveniles of Wheat Seed GALL Nematode *Anguina tritici*

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ABSTRACT

This study consisted of two in - vitro bioassay, in the 1st one four medicinal plants were selected to test nematicidal activity of their aqueous extracts (A.E) against 2nd stage juveniles (Js2) of wheat seed gall nematode *Anguina tritici* included: leaves of Mint, Datura, Eminium (Zilikeraba, Kary) and Pomegranate peels. Results revealed that the highest nematode mortality (92.9%) was recorded in the A.E. of Mint leaves, while the lowest (76.7%) in A.E. of Datura leaves regardless, their mixing ratio with distilled water (D.W.). Nematode mortality significantly increased by increasing mixing ratio. and in general the highest nematode mortality (100 %) was recorded by A.E. of Eminium leaves after a week of immersing J2 in its higher mixing ratio (8ml A.E.: 10 ml D.W.) with no significant difference compared to A.E. of mint leaves at its last two mixing ratio (4 ml A.E.: 10 ml D.W. and 8 ml A.E.:10 ml D.W.), whilst the lowest nematode mortality (66.1%) was recorded by the same plant (Eminium) at its lower mixing ratio (1ml A.E. : 10 ml D.W.). In the 2nd in – vitro bioassay Velum prime as nematicide, Albendazole as anthelmintic drug and BM Root Pan as bio-control agent were selected to test their nematicidal acivity against Js2 of A. tritici. Results showed that the highest nematode mortality (46.5%) was recorded by Velum prime with significant difference compared to the other control agents meanwhile, the lowest mortality (31.25%) was reported by BM-Root-Pan regardless their concentrations. In general the highest nematode mortality (78 %) was found when they were immersed in Velum prime at concentration 5ppm with significant difference compared to the other concentrations for the same nematicide and for the other control agents followed by Albendazole drug (62%.) at concentration 3mg whilst, the lowest nematode mortality was recorded by BM - Root – Pan at concentration 0.1x $10^6$.



## 1. Introduction

Wheat seed gall nematode *Anguina tritici* (Steinbuch, 1799; Filipjev, 1936) *(Anguinidae, Rhabditida*) the cause of ear-cockle disease in wheat discovered for the first time by John Needham in England in 1743 (Perry and Moens, 2006). Bhatti, et al., (1978) stated that the earliest known of wheat diseases was ear cockle disease caused by wheat seed gall nematode A. tritici. It is a serious aerial disease that permanently damages wheat in tropical and sub-tropical regions (Kort, 1972). This pest is still common in Eastern Europe, parts of Asia, and Africa, and it is present wherever wheat is planted (Agrios, 2005). In Iraq, the first record of ear-cockle disease was reported by Rao in 1921.

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## 2. Literature review

Throughout most of the areas in Iraq where wheat is grown, this nematode remains an important pest by 22.9 to 45% on mexipak c.v. of wheat (Al-Beldawi et.al.1974) and up to 75% on the same c.v.in Duhok province in 1989 (Stephan and Antoon, 1990). This nematode has been detected in the USA included states of California, Georgia, Maryland, New York, North and South Carolina, Virginia and West Virginia (CABI,2005). According to Ami, et.al., (2004) the percentage of infestation by seed galls reached a maximum of 50% in bread wheat in Bashika - northern of Iraq. Ami and Guri ( 2023) reported the presence of wheat seed gall nematode  tritici in the silos and certain wheat fields in Duhok province / Kurdistan Region – Iraq, where they discovered that samples of wheat impurities were infested with seed galls by 66 % as the highest percentage in silo of Faidia during 2020 and by 6% as the lowest percentage in the silo of Zakho during the same year, they also recorded the highest disease incidence (34.6%,) in the wheat fields of Akre , while the lowest (2%) in the wheat fields of Semel. According to Paruthi and Bhatti (1988), ear-cockle disease lowered wheat consumption and market prices. It also significantly reduced the protein and gluten contents of flour made from wheat that has seed gall infestations (Mustafa, 2009). The use of plant extracts with nematocidal properties as alternative to synthetic pesticides for controlling plant parasitic nematodes is becoming important. In recent years, studies on this subject have increased quickly in the Mediterranean coast (Ntallie et al., 2011; Andres et et al., 2012 and Ntallie et al., 2020). Results of Bioassay showed that fungicides Vitavax, Divident, Dithane and herbicide “Granstar”, caused mortality to J2 of A. tritici reached to 89.125% after a week of j2 immersing in 8 ppm Dithane, Furthermore, spore suspension of Biocont-T Trichoderma harzianum (19×106 spores/ml) caused 53.9% mortality of J2 (Taher,2012). Recently, the highest mortality (48.61%) of J2 of A. tritici was found in the aqueous extract of chinaberry fruit while, the lowest (43.7 5%) in the aqueous extract of tobacco leaves and J2 mortality increased with increasing concentrations of the aqueous extract (Guri, 2023). This study aimed to implement two bioassay experiments, the first targeted to test the nematicidal activity of the aqueous extracts of selected plant parts namely: leaves of each of Mint (*Mentha spicata* L.), Datura (*Datura stramonium*) and Zilikeraba (Kary) *Eminium spiculatum* and Pomegranate peels (*Punica granatum*) against vitality of 2nd stage juveniles ( J2 ) of wheat seed gall nematode *A. tritici* while, the 2nd aimed to test nematicidal activity of two chemicals included Velum prime as a nematicide and Albendazole as anthelmintic drug compared to the BM Root Pan as bio – control agent against vitality of Js2 of the same nematode species.

## 3. Materials and methods

### 3.1 Selected plants for the 1st experiment

Four medicinal plants were selected for this study according to their component of active ingredients (Table, 1) included: Mint (*Mentha spicata L.,* Lamiaceae), Datura (*Datura stramonium,* Solanaceae), Eminium (Zilikeraba, Blume, Kuntze, Kary) (*Eminium spiculatum,* Aracea), and Pomegranate peel (*Punica granatum,* Lythraceae).Where Mint was collected from the home garden while, Datura which grows naturally, as a weed plant was collected from the college fields. As for Eminium and .pomegranate, they were purchased from local markets. Leaves for each of Mint, Datura and Eminium (Zilikeraba, Blume, Kuntze, Kary) and peels of pomegranate were used for extraction their chemical components using distilled water as a solvent. Plant materials were dried in the shade after being brushed individually on pieces of polyethylene in the laboratory with constant stirring daily to avoid rotting.

Table 1. Plant name and their content of active ingredients

| Plant name | The main active ingredients (a.i.) |
|---|---|
| Mint | Menthol, Terpenoids, and Flavonoids (*https://en.wikipedia.org/wiki/Pepperm int# Chemical constituents*) |
| Datura | Tropane alkaloids atropine, hyoscyamine, and scopolamine, (*https://en.wikipedia.org/wiki/Datura_stramonium#Toxicity)* |
| Eminium (Zilikeraba, Kary) | Toxic compound: Calcium oxalate crystals. In addition to other active compounds such as Alkaloids, Saponins and Flavonoids (*http://www.aec.org.sy/poisonous_plants/poisonous_plants_app.php?id=45)* |
| Pomegranate peels | Phenolic acids, flavonoids, and tannins (*https://www.hindawi.com/journals/jfq/2020/8850339/ )* |

The plant parts were ground with an electric grinder, then the powder was kept in polyethylene bags individually until used. Nine gm of each powder was individually immersed in 90 ml of distilled water (D.W.), then after 3 days the mixture was heated in water bath at 60 °C for 2 hrs and after cooling they were filtered separately by Whatman No.1 filter paper, the leachates were placed individually in a glass beaker of 90 ml size which was sealed and wrapped with cellophane paper to protect materials from light and prevent their oxidation then they were kept in the refrigerator to use them later.

### 3.2 Required materials for the 2nd experiment

Velum Prime® SC400 (purchased from local markets) as nematicide with active ingredient (a.i ) 400g / L Fluopyram; ($C1_6$ H11Cl$F_6$N2O) (N-{2-[3-Chloro-5-(trifluoromethyl) pyridine-2-yl]ethyl}-2-(trifluoromethyl) benzamide. Formulation: Suspension Concentrate, Albendazole 200 mg / 5ml as anthelminthic drug (purchased from the pharmacy) which is usually used against human parasitic worms.BM-Root Pan that contains four bacterial species including: *Bacillus megaterium, Bacillus subtilis*, *Paenibacillus polymexa* and *Pantoea agglomerans* and as a rate of 1 x $10^7$ bacterial cells/ml produced by Tarım A.Ş.Turkish – British Company.

### 3.3 Preparation of nematode suspension

One of the wheat seed galls (Fig. 1) that have been brought with infested grain wheat samples from silo of Zakho city and have been formed due to wheat infection by wheat seed gall nematode *A. tritici* was placed in D.W. using Petri dish for the period of 2 days, then the gall was opened with the aid of 2 sterilized needles for releasing 2nd stage juveniles (Js2) (Fig.2) from the gall.

Figure 1. Healthy grains wheat (left) compared to wheat seed galls caused by wheat seed gall nematode A. tritici (right)

Nematode population in the resulting suspension was calculated using modified counting dish where 10 ml of the nematode suspension was placed in it and with the aid of stereomicroscope nematode number was calculated. This operation was replicated 5 times, and it was found that each 2 ml of nematode suspension contained 46 ± 4 Js2 nematodes.

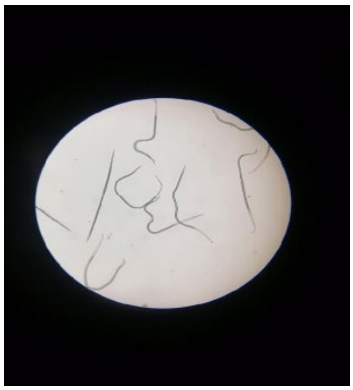

Figure 2. 2nd stage juveniles of wheat seed gall nematode *A. tritici* used in both experiments

### 3.4 In vitro bioassay study

Bioassay of Js2 of A. tritici against aqueous extract of selected plants: Five concentrations for each aqueous extract using dilution ratio (ml of plant extract: ml of D.W.) were prepared from the leachate of the extract separately involved 0.0 :10, 1:10, 2: 10, 4: 10, and 8: 10 ml where 0.0: 10 included immersions of juveniles in D.W. Three ml of the aqueous extract for each dilution ratio and 2ml of nematode suspension with 1ml of D.W. (to reduce the dilution ratio to half) were placed individually in each plastic Lab. container where 0.0 included.

### 3.5 Preparation concentrations of control agents

Five concentrations for each control agent were prepared including control treatment (0.0 ) as follow: Velum prime: 1ml of this nematicide was added to 99 ml of D.W. then the following concentrations were prepared : 2,3,4 and 5 ppm; lBM Root Pan :1m of this bio - control agent was added to 99 ml of D.W. to prepare the following concentrations : 0.1x $10^6$, 0.2x $10^6$, 0.3x $10^6$ and 0.4 x $10^6$; Albendazole C12H15N3O2S ( brand name Albenza): 0.5 mg.a.i., 1 mg.a.i., 2 mg.a.i. and 3 mg.a.i after dissolving 2 ml of the drug in 99 ml of D.W. To obtain these concentrations double concentration were prepared in each case due to the same amount of Js2 nematode suspension with D. W. was added to each Lab container to get the requested concentration.

### 3.6 In vitro bioassay study

Immersion of 2 ml of juvenile suspension in 4 ml of D.W. containers were covered and left under laboratory conditions (16 ± 5 ℃). Nematode mortality was calculated after a week. Mortality percentage was corrected according to the following equation mentioned by Ami (1998). Corrected mortality percentage=100-100 × (number of livings J2 in the treatment / number of livings J2 in the control).

Dead juveniles were discriminated by: Straightening of juveniles and turning brown in colour, and Juveniles lose their movement after being submerged in water for two - three hrs.

### 3.7 Experimental design and data analysis

The first experiment consisted of 16 treatments (4 plant extracts × 4 concentrations) and the 2nd experiment consisted of 12 treatments (3 control agents × 4 concentrations) where control treatment was excluded after extracting mortality percentage of Js2. Each treatment replicated three times and implemented as factorial experiment in Completely Randomized Design (CRD). Results statically analysed and tested by Duncan's Multiple Range Test (DMRT) $p \leq 0.05$.

## 4. Results and discussions

Results showed that the aqueous extracts (A.E.) of all tested plants were effective in death of Js2 of wheat seed gall nematode *A. tritici* (Table, 2), where the highest Js2 mortality (92.9 %) was recorded by the A.E. of mint plant with significant superiority compared to the other plant extracts, while the lowest nematode mortality (76.7 % ) was recorded by the aqueous extract of Datura plant, as well as nematode mortality increased by increasing extract concentration and it was found that the highest nematode mortality (92.6%) was observed at the higher dilution ratio (8 ml A.E: 10 ml D.W.) with significant difference compared to the other concentrations, meanwhile the lowest nematode mortality (76.2%) was observed at the lowest dilution ration (1 ml A.E.: 10 ml D.W).

Table 2. Mortality of 2nd stage juveniles of wheat seed gall nematode *A. tritici* as affected by some plant extracts

| Common and scientific name of plants from which extract was prepared | Dilution ration (P. E.: 10 ml D.W.) | Js2 mortality (%) | Effect of dilution ratio | Effect of plant extract |
|---|---|---|---|---|
| Mint (*Mentha spicata* L) | 1 | 83.7 bcd | | 92.9 a |
| | 2 | 89.5 b | | |
| | 4 | 99.2 a | | |
| | 8 | 99.2 a | | |
| Pomegranate peels (*Punica granatum*) | 1 | 86.4 bc | | 87.2 b |
| | 2 | 86.4 bc | | |
| | 4 | 87.1 bc | | |
| | 8 | 88.8 b | | |
| Datura (*Datura stramonium*) | 1 | 68.5 gh | | 76.7 d |
| | 2 | 75.5 ef | | |
| | 4 | 80.6 de | | |
| | 8 | 82.3 cd | | |
| Zilikeraba (Kary) (*Eminium spiculatum*) | 1 | 66.1 h | | 80.8 c |
| | 2 | 72.6 fg | | |
| | 4 | 84.6 bcd | | |
| | 8 | 100 a | | |

**Each value followed by the same letter (s) in each column does not differ significantly according to Duncan's multiple range test ($p \leq 0.05$). *Any value of the interaction between plant extracts and concentrations is a mean of 3 replications.*

Additionally, statistical analysis demonstrated that the interaction between plant extracts and their concentrations was significant in its effect on J2 mortality and in general, the highest nematode mortality (100 %) was recorded by Eminium (Zilikeraba, Kary) plant after a week of immersing juveniles in it at the higher dilution last two dilution ratio (4 ml A.E.:10 ml D.W. and 8 ml A.E:10 ml D.W.), whilst the lowest ratio ( 8 ml A.E.:10 ml D.W) with no significant difference with the extract of mint plant at its

nematode mortality (66.1%) was recorded by the same plant at its lower dilution ratio ( 1ml A.E. : 10 ml D.W.). Results of the present study agree with those of the previous studies that the aqueous extracts of different plant parts showed nematocidal activity against *M .javanica* (Dawar et al., 2007 and Hassan et al.,2015), *Meloidogyne spp*. (Kouamé, et al. 2021) and *M. arenaria* (Abdulgahar, 2023) and also with another study conducted by Guri (2023) who indicated that the aqueous extracts of different plant parts developed a nematocidal activity against Js2 of wheat seed gall nematode *A.tritici.*

This effect is attributed to the nematocidal activity of the active ingredients or toxic materials present in these plant parts, where they are biologically active and it is clear that the active ingredients such as calcium oxalate crystals, in addition to the other active compounds such as alkaloids, saponins and flavonoids in E. *spiculatum* was more effective bionematicidal compounds than the other active ingredients of the other plants such as menthol, terpenoids and flavonoids in Mint leaves and Tropane alkaloids atropine, hyoscyamine, and scopolamine in Datura plant leaves and Phenolic acids, flavonoids and tannins in Pomegranate peels.

The nematocidal activity and mechanism effect of these plant extracts may return back to their effect on the respiratory function of nematode juveniles, resulting in their paralysis and eventual death, in addition to their ability in modifying membrane permeability which means disrupting cytoplasmic membrane of nematode cells and their functional groups and tampering with the structure of protein enzyme by causing degradation and denaturation of protein and enzyme inhibiting or interfering with the enzymatic reactions of energy metabolism. This opinion is consistent with what has been mentioned by Knoblock et al. (1989) or may have acetylcholine esterase inhibiting action.

Regarding results of the 2nd experiment statistical analysis (Table 3) revealed that the highest mortality (46.5%) of Js2 of wheat seed gall nematode *A. tritici* was recorded by Velum prime as chemical nematicide, regardless its concentrations with significant difference compared to the other control agents followed by Albendazole as anthelmintic drug where Js2 mortality reached 40.25%, meanwhile

Table 3. Mortality of Js2 of wheat seed gall nematode *A. tritici* as affected by different concentrations of Velum, BM Root Pan and Abendazolel

| Control agent | Concentration (ppm) | Js2 mortality (%) | Effect of the control agent |
|---|---|---|---|
| Velum prime (Nematicide) | 2 ppm | 26 g | 47.5 a |
| | 3 ppm | 37 e | |
| | 4 ppm | 49 c | |
| | 5 ppm | 78 a | |
| BM Root Pan (Bio- control agent) | $0.1x\ 10^6$ | 19 h | 31.25 c |
| | $0.2x\ 10^6$ | 25 g | |
| | $0.3x\ 10^6$ | 34 e f | |
| | $0.4x\ 10^6$ | 47 cd | |
| Albendazole (Anthelmintic drug) | 0.5 mg.a.i. | 23 g h | 4.25 b |
| | 1 mg.a.i. | 32 f | |
| | 2 mg.a.i | 43 | |
| | 3 mg.a.i | 63 | |

**Each value followed by different letter in the same column does not differ significantly according to the Duncan multiple range test ($P \leq 0.05$).*
**Any value of the interaction between control agents and concentrations is a mean of 3 replications.*

the lowest nematode mortality (31.25%) was reported when nematode Js2 were immersed in BM Root Pan as bio -control agent. In general, the highest nematode mortality (78 %) was found when they were immersed in Velum prime nematicide at concentration (5ppm) with significant difference compared to the other concentrations either for the same nematicide or for the other control agents followed by Albendazole drug at concentration 3mg a.i. with mortality 62 % whilst the lowest nematode mortality (19 %) was recorded by BM - Root – Pan at concentration 0.1x $10^6$.

Nematode mortality is attributed to the influence of control agent which differ from each other in their mechanism effect and thus nematode mortality caused by Velum Prime is return back to its positive effect on nematode juveniles, since the active ingredient of this nematicide, fluopyram, is a member phenyl-benzamide group, it inhibits the respiratory chain in the mitochondria by preventing the production of adenosine triphosphate, which is the fundamental compound that provides energy in the cell, and this results in the cessation of vital processes, paralysis nematodes and their eventual death.

According to Haydock (2013), Faske and Hurd (2015) and Alhayali (2021), it is a non- selective pesticide that impacts through contact and digestive system, The effect of Albendazol on nematode vitality is ascribed to the influence of this active ingredient, which reduce ability of the worms to produce energy, causing degenerative modification in the intestinal cells, that finally causes immobilization and death of the parasite. Degenerative alterations in the endoplasmic reticulum, the germinal layer's mitochondria, and the ensuing release of lysosomes lead to a reduction in the synthesis of adenosine triphosphate (ATP), the energy needed for the helminth to survive (https://go.drugbank.com/drugs/DB00518). Regarding BM -Root Pan this bio-control agent contains races of several bacteria included: *Bacillus megaterium, Pantoea agglomerants, Paenibacillus polymexa* and *Bacillus subtili,* where these species can release toxins that come from bacterial secondary metabolism, and as shown by El-Nagdi and Youssef (2004),Carneeiro et al. (1998), and Al-Hayali (2021), these toxins play a critical role in paralyzing Js2of wheat seed gall nematode.

## 5. Conclusions

Results of the 1st experiment revealed that all aqueous extract of the selected plant parts have a nematocidal effect and it was shown that Eminium and Mint leaves at their higher concentrations (8 ml A.E.:10 ml D.W.) were more effective on nematode Js2 vitality compared to the aqueous extract of Datura plants and Pomegranate peels. Results of the 2nd experiment indicated that the highest nematode mortality was found when they immersed in Velum prime nematicide at concentration 5 ppm with significant difference compared to the other concentrations either for the same nematicide or for the other control agents, whilst the lowest nematode mortality was recorded by BM - Root – Pan as bio - control agent at its low concentration ( 0.1x $10^6$ ).

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