Saudi Journal of Biological Sciences 28 (2021) 4442–4445

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Effect of some fertilizers on hatching of cereal cysts nematode, *Heterodera avenae*

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ARTICLE INFO

*Article history:*
Received 8 May 2020
Revised 13 April 2021
Accepted 14 April 2021
Available online 24 April 2021

*Keywords:*
Cereal cyst nematode
*Heterodera avenae*
Hatching
Fertilizers

ABSTRACT

Experiments were conducted in laboratory and pot conditions to determine the effects of urea, diammonium phosphate (DAP), single super phosphate (SSP), muriate of potash (MOP) and zinc sulphate ($ZnSO_4$) on hatching of *Heterodera avenae*. Two concentrations of each fertilizer were tested in lab for which 10 cysts and 5 ml of each concentration were taken in 5 cm diameter Petri plates. Observations were recorded at weekly intervals up to six weeks. Urea, DAP, SSP and MOP inhibited hatching and $ZnSO_4$ increased it. After six weeks, hatching was least (5.45%) in higher dose of urea and greatest (46.9%) in higher dose of $ZnSO_4$. In pot experiment, two doses of urea and single dose of SSP, MOP, and $ZnSO_4$ were applied in *H. avenae*-infested soil and WH-1105 wheat was sown. Observations on nematodes in roots, soil and remaining cyst contents were recorded 40 days after sowing. Among all the fertilizers, least nematodes in soil and roots were found at higher dose of urea and greatest number in $ZnSO_4$.



## 1. Introduction

Cereal cyst nematodes have worldwide distribution and are documented to be of economic importance on wheat mainly in light textured soil and where predominately cereal monoculture is practiced (Nicol and Rivoal, 2008). Organic and synthetic fertilizers had a suppressant effect on nematodes (Kaplan and Neo, 1993). Application of fertilizers affects nematode populations indirectly by increasing the nematode feeding or by providing nutrition to compensate the plant from the nematode feeding (McIntoch et al., 1999). On the other hand, nutrient deficiency may make the plant weak and more susceptible to nematode attack (Melakeberhan et al., 1997). The direct effect of fertilizers on nematodes may alter nematode behavior and reproduction which may result in either a decrease or increase of the population. The effectiveness of fertilizers in changing nematode population depends on the fertilizer components and their active ingredients (Gruzdeva et al., 2007). The fertilizer components may be lethal directly to nematodes or they may alter both pH and salinity of the soil which in turn affect nematode populations (Oka and Pivonia, 2002; Tenuta and Ferris, 2004). Some studies have shown that inorganic fertilizers affect the hatching of nematode species (Tefft and Bone, 1984; Clarke and Shepherd, 1966).

Some nutrients have been reported to inhibit nematode hatching (Habash and Al-Banna, 2011; Zhen-yue et al., 2013) while others to act as stimulant (Behm et al., 1995; Tefft and Bone, 1984). *Heterodera avenae* is a serious pest of cereals-wheat, barley and oat (in European conditions), however rice is also a cereal but *H. avenae* won't affect it. Unlike *Globodera* spp., its hatching is not dependent on root exudates of host plants. But some factors do affect its hatching which continues for more than two months and all the eggs do not hatch in one year (Kanwar and Bajaj, 2011; Clarke and Shepherd, 1966). Fertilizers are commonly applied to all crops/wheat according to their requirement and soil fertility status. These fertilizers having some suppressive/toxic, stimulating/attractant and neutral effect on plant diseases or pathogens (Dordas, 2008) so, present study examined the effects of synthetic fertilizers on hatching of cereal cyst nematode in laboratory and pot conditions.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.04.039
1319-562X/

## 2. Materials and methods

### 2.1. Location

The experiments were conducted in laboratory and pot conditions during rabi season of 2016–17 in the screen house of Department of Nematology, Choudhary Charan Singh Haryana Agricultural University, Hisar, Haryana (India) which has situated at Latitude – 29°10′ N, Longitude – 75° 46′ E, Altitude – 215.2 m.

### 2.2. Experimental procedure

Laboratory experiment was done in 5 cm diameter Petri plates. Urea, di-ammonium phosphate (DAP), single super phosphate (SSP), muriate of potash (MOP) and zinc sulphate ($ZnSO_4$) were included in the study. These fertilizers are commonly used by farmers as sources of nitrogen, phosphorus, potash and zinc, respectively, in India. The recommended doses of fertilizers in wheat NPK and Zn mg per kg soil were converted in to the ppm for the laboratory studies. Required PPM concentration of each fertilizer was prepared by mixing calculated fertilizers amount in one liter of water. Two concentrations of each fertilizer at single and double dose of recommended dose for wheat crop were prepared. Ten hand-picked cysts were placed in each Petri dish containing 5 ml of each fertilizer's concentration. Cysts placed in water served as control. Each treatment was replicated four times. The cysts were incubated for six weeks at room temperature (December 2016 to January 2017, temp. 15–25 °C). The hatched $J_2$ were counted at weekly intervals, up to six weeks. Un-hatched cyst contents were estimated by crushing the cysts under a dissecting microscope and per cent hatching (larval emergence) was calculated.

$$\%\text{Larval emergence} = \frac{\text{Total larval emergence}}{(\text{Average cyst content} \times 10) + \text{Totallarval emergence}} \times 100$$

In second experiment, *H. avenae* susceptible wheat cultivar WH-1105 (Anonymous, 2014) was sown in 15 cm diameter earthen pots having 1 kg soil. Initial inoculum was 13 eggs and $J_2$/g soil (327 eggs and juveniles cyst$^{-1}$). The soil was got analyzed from Department of Soil sciences, CCS HAU, Hisar. It had sand, silt and clay 89.7, 4.5 and 5.8 per cent, respectively, and initial level of N = 116 kg/ha, P = 15 kg/ha, K = 165 kg/ha and Zn = 1.31 ppm. The recommended doses of NPK and Zn in wheat has 150, 60, 60 and 25 kg ha$^{-1}$ (163 mg urea, 187.5 mg SSP, 50 mg MOP and 59.5 mg $ZnSO_4$ kg soil$^{-1}$) respectively, in Haryana, India. Recommended doses of urea, SSP, MOP, $ZnSO_4$, combination of all fertilizers, half dose of urea (split dose of urea applied at the time of sowing) and without fertilizers were taken as treatment (Table 2). The treatments with three replications were arranged in completely randomized design on a bench of screen house. After 40 days of sowing observations were recorded on soil and root population, and cyst contents. For recording root population, roots were stained by acid fuchsin-lactophenol (McBeth et al., 1941). Cysts from soil were extracted by Cobb's method (Cobb, 1918) and juveniles by modified Baermann funnel method (Schindler, 1961).

### 2.3. Statistical analysis

The data were analyzed using completely randomized design by IBM SPSS statistics version 22. Data were subjected to analysis of variance (ANOVA) and the comparisons in treatments were made by critical difference (CD) at 5 per cent level of significance.

## 3. Results

### 3.1. In vitro effect of fertilizers on hatching

Data (Table 1) revealed that in first week, there was no hatching in urea, DAP, SSP and MOP but a few larvae hatched in $ZnSO_4$ and water. In second week, hatching was significantly less in all fertilizers than water, except in both doses of $ZnSO_4$. Maximum hatching (119.25) was observed in $ZnSO_4$ at 120 ppm which was significantly higher than the hatching in control (74.75). Minimum hatching was observed in higher concentration of urea. During third week, $ZnSO_4$ stimulated hatching while other fertilizers inhibited it. Hatching was maximum (182.25) at higher dose of $ZnSO_4$.

In all the treatments maximum hatching occurred in fourth week. At this time, maximum hatching was recorded in 120 ppm $ZnSO_4$ and minimum in urea at 160 ppm. In fifth and sixth weeks hatching trend remained same. After six weeks, average unhatched cyst contents were highest in higher concentration of urea and lowest in higher concentration of $ZnSO_4$. It is because urea suppressed hatching and more contents were left in the cysts. Contrary to this, due to stimulatory effect of $ZnSO_4$, more larvae hatched and less contents were left in this treatment.

Hatching was significantly more in both concentrations of $ZnSO_4$ as compared to control. At 120 and 60 ppm $ZnSO_4$, 46.65 and 39.31% hatching occurred respectively, in comparison to 32.55% in water (Fig. 1). In all other fertilizers, at both concentrations, weekly as well as total hatching was much less than control showing their inhibitory effect. This trend was seen from beginning through the experimental period.

**Table 1**
Effect of fertilizers on hatching of *Heterodera avenae* under laboratory conditions.

| Treatments | No. of hatched juveniles from 10 cysts | | | | | | Total | Left over eggs cyst$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | | |
| Urea 80 ppm | 0.00$^{a}$ | 8.75$^{b}$ | 26.00$^{b}$ | 57.00$^{a}$ | 35.25$^{a}$ | 40.25$^{a}$ | 167$^{b}$ | 221.75$^{cd}$ |
| Urea 160 ppm | 0.00$^{a}$ | 4.25$^{a}$ | 19.50$^{a}$ | 45.00$^{b}$ | 32.25$^{a}$ | 35.50$^{a}$ | 136$^{a}$ | 234.75$^{d}$ |
| DAP 65 ppm | 0.00$^{a}$ | 23.25$^{d}$ | 44.25$^{d}$ | 79.50$^{cd}$ | 47.00$^{b}$ | 53.50$^{b}$ | 247$^{d}$ | 216.5$^{cd}$ |
| DAP 130 ppm | 0.00$^{a}$ | 17.25$^{c}$ | 37.50$^{c}$ | 71.25$^{c}$ | 43.25$^{b}$ | 48.25$^{b}$ | 217$^{c}$ | 220.5$^{cd}$ |
| SSP 187 ppm | 0.00$^{a}$ | 37.75$^{f}$ | 59.00$^{e}$ | 86.00$^{de}$ | 59.00$^{c}$ | 69.25$^{d}$ | 311$^{e}$ | 219.25$^{cd}$ |
| SSP 375 ppm | 0.00$^{a}$ | 32.25$^{e}$ | 55.75$^{e}$ | 94.50$^{e}$ | 54.50$^{c}$ | 62.75$^{c}$ | 299$^{e}$ | 217.5$^{cd}$ |
| MOP 50 ppm | 0.00$^{a}$ | 50.00$^{g}$ | 77.25$^{g}$ | 122.75$^{f}$ | 76.50$^{e}$ | 82.25$^{f}$ | 409$^{g}$ | 206.0$^{c}$ |
| MOP 100 ppm | 0.00$^{a}$ | 46.00$^{g}$ | 71.00$^{f}$ | 113.50$^{f}$ | 70.25$^{d}$ | 77.50$^{e}$ | 378$^{f}$ | 216.75$^{cd}$ |
| $ZnSO_4$ 60 ppm | 3.75$^{c}$ | 99.25$^{i}$ | 156.50$^{i}$ | 208.00$^{h}$ | 126.25$^{g}$ | 141.75$^{h}$ | 732$^{i}$ | 113.25$^{a}$ |
| $ZnSO_4$ 120 ppm | 5.50$^{d}$ | 119.25$^{j}$ | 182.25$^{j}$ | 254.25$^{i}$ | 148.75$^{h}$ | 161.50$^{i}$ | 866$^{j}$ | 98.75$^{a}$ |
| Water (control) | 2.50$^{b}$ | 74.75$^{h}$ | 134.75$^{h}$ | 190.50$^{g}$ | 115.50$^{f}$ | 127.00$^{g}$ | 642$^{h}$ | 133.0$^{b}$ |

Data are means of four replications randomized completely. Data were subjected to analysis of variance (ANOVA) with IBM SPSS statistics version 22. Values with same letters in a column denote non-significant difference ($P \leq 0.05$).

**Table 2**
Effect of fertilizers on hatching of *Heterodera avenae* in pots grown with wheat.

| Treatments | Nematodes/200 cc soil | Nematodes/root system | Left over contents per cyst |
|---|---|---|---|
| No fertilizer (control) | 185.3$^{e}$ | 161.0$^{d}$ | 113.7$^{a}$ |
| Urea 163 kg /ha | 68.3$^{ab}$ | 89.7$^{ab}$ | 212.3$^{d}$ |
| Urea 326 kg /ha | 60.7$^{a}$ | 79.0$^{a}$ | 229.0$^{e}$ |
| SSP 375 kg /ha | 95.0$^{bc}$ | 110.0$^{bc}$ | 182.0$^{c}$ |
| MOP 100 kg /ha | 106.0$^{c}$ | 119.3$^{c}$ | 174.7$^{c}$ |
| $ZnSO_4$ 119 kg /ha | 142.3$^{d}$ | 147.0$^{d}$ | 132.3$^{b}$ |
| Urea (full dose) + SSP + MOP + $ZnSO_4$ kg /ha | 108.7$^{c}$ | 111.3$^{bc}$ | 186.0$^{c}$ |

Data are means of three replications randomized completely. Data were subjected to analysis of variance (ANOVA) with IBM SPSS statistics version 22. Values with same letters in a column denote non-significant difference ($P \leq 0.05$).

### *3.2. Effect of fertilizers on hatching under pots*

Data (Table 2) revealed that all the fertilizers treatment suppressed the hatching from cysts, except $ZnSO_4$. In all the fertilizers treatments, juveniles in soil were less than soil without fertilizers and this number was maximum in $ZnSO_4$ and lowest in higher dose of urea. $ZnSO_4$ increased the no. of juveniles in soil and roots but it was less than untreated check. Among all the treatments, maximum juveniles in wheat roots were recorded in water (161.0) though this number was not statistically significant compared to $ZnSO_4$. Average cyst content left in soil was affected by presence of fertilizers. It was lowest in $ZnSO_4$ and highest in urea at 326 kg/ha. In $ZnSO_4$, hatching was more than the combined application of all fertilizers but less than control. It is so because in combined application, stimulatory effect of $ZnSO_4$ was mitigated by suppressive effect of other fertilizers. Hence interactive effect of different micro and macro nutrients in soil affecting the nematode hatching and survival play important role and needs to be studied.

## 4. Discussion

In our laboratory study among all fertilizers, both the concentrations of $ZnSO_4$ stimulated the hatching from cysts. In pots, $ZnSO_4$ increased the no. of larvae in soil and roots over other fertilizers though it was less than untreated check. In pots we used only recommended dose of $ZnSO_4$ which may be the reasons for less effect than lab. Neutral effect of $ZnSO_4$ in pot conditions might be due to the reason that soil is a buffer medium and nematodes are not in direct contact of fertilizers as in lab experiment. Clarke and Shepherd (1966) hypothesized that soil applications of zinc would not be practical for management of cyst nematodes because zinc cations are readily adsorbed to clay particles in the soil, and free zinc cations are necessary for stimulation of egg hatch. Present results are similar with the studies of Krauskopf (1972) who found that zinc salts are effective stimulants for hatching at concentrations that approximate soil levels.

Our lab studies showed that zinc sulphate increased the larval emergence from cyst at both the doses. Similar results were obtained by Behm et al. (1995) who reported that *in vitro* egg hatching of *Heterodera glycines* in reagent-grade zinc sulfate and zinc chloride, and fertilizer-grade zinc sulfate was significantly greater than hatching in deionized water, whereas zinc chelate fertilizer significantly inhibited egg hatching relative to deionized water. Commercial zinc chelate fertilizer inhibited hatching. It is likely that the hatch inhibition was caused by the chelating agent, although their investigation did not examine the effects of the chelating agent alone. They found that zinc compounds significantly affected *H. glycines* egg hatching *in vitro*, but had no effect on hatching in natural soils. In present study only fertilizer grade zinc sulphate has been tested and found that no affects in pot conditions. Stimulatory effect of zinc salts was also reported on egg hatching in soybean cyst nematode, *H. glycines* (Clarke and Shepherd, 1966; Tefft and Bone (1984) and *H. avenae* (Zhen-yue et al., 2013). Egg hatching of the soybean cyst nematode, *Heterodera glycines*, was not affected by millimolar concentrations of calcium sulfate or calcium chloride. However, zinc chloride and zinc sulfate caused strong and moderate increases in hatching, respectively (Tefft and Bone, 1984).

Results indicated that the maximum suppression of larval hatch as shown by their presence in soil and roots was observed in urea. Application of ammonium fertilizers has shown nematicidal effect

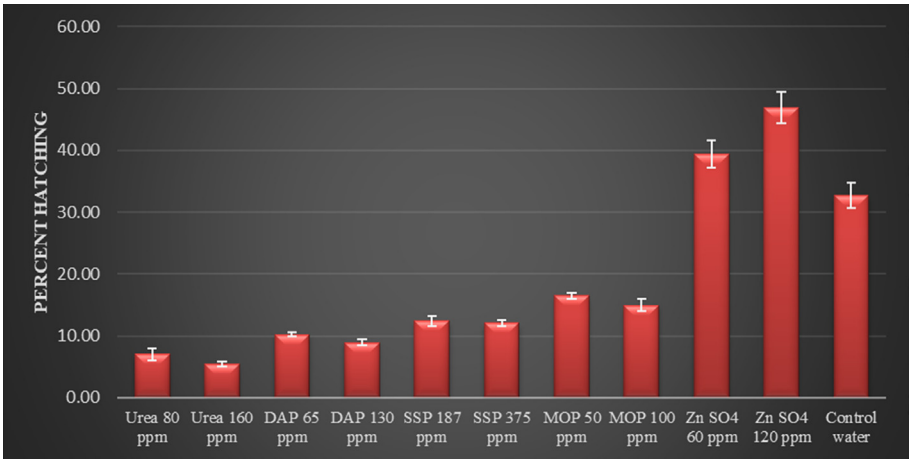


**Fig. 1.** *In vitro* effect of fertilizers on hatching of *Heterodera avenae*.

on plant-parasitic nematodes (Sudirman and Webster, 1995; Rodriguez-Kabana et al., 1982; Zhen-yue et al., 2013). Egg-hatching and viability of *M. javanica* larvae were greatly reduced when $NH_4Cl$, $(NH_4)^2 SO_4$ and $NH_4NO_3$ were used at elevated levels of EC, and there was a reduction in rate of nematode infection after their use on cucumber under greenhouse conditions (Karajeh and Al-Nasir, 2012).

In present study higher dose of urea was more effective in reducing *H. avenae* larvae in soil and roots. Urea is readily converted to ammonia, a conversion which is necessary for urea to be effective both as fertilizer and as nematicide (Siddiqui et al., 2001) and it is dosage dependent (Rodriguez-Kabana and King, 1980). In wheat crop, SSP, MOP and $ZnSO_4$ are applied at sowing time while urea is given in 2–3 split doses depending on soil type. Maximum hatching in *H. avenae* takes place in 30–40 days, hence split application of nitrogenous fertilizers (urea) may not be effective in inhibiting its hatching.

Single super phosphate, di-ammonium phosphate and muriate of potash also suppressed hatching of *H. avenae* as indicated in our lab and pot studies. Saeedizadeh et al. (2020) tested four fertilizers including a chemical fertilizer (NPK), an organic fertilizer (Humic acid), a semi-organic fertilizer (Agro-health) and a bio-fertilizer (Azotobarvar-1) *in vitro* and observed that the fertilizers increased second stage juveniles (J2s) mortality and decreased egg hatching of root-knot nematode. Zhen-yue et al. (2013) also reported that Zn significantly stimulated the hatching of *H. avenae*. In their studies zinc did not cause mortality of secondary stage juveniles but other tested elements increased mortality as compared to control. Hatching suppressive effect of the fertilizers can be directly beneficial to crop for protection against nematode. Hatching stimulating effect of $ZnSO_4$, can be used for obtaining more larvae of *H. avenae* from cysts for laboratory studies. Higher doses of zinc fertilizers resulting in more hatching can be exploited for management of *H. avenae* through chemicals and trap crops etc.

## 5. Conclusion

Synthetic fertilizers urea, single super phosphate, di-ammonium phosphate, muriate of potash inhibited hatching of cereal cyst nematode in lab as well as pot conditions. Zinc sulphate stimulated it in lab but showed no effect in pot conditions. All these fertilizers are widely used in agriculture and can easily be used to control nematode without adversely affecting agricultural land or harming human health. Also, the hatching stimulating agent like zinc sulphate can be exploited as management practice for cereal cyst nematode.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

Authors are thankful to Professor and Head, Department of Soil Science, CCS HAU, Hisar, Haryana for analysis of soil samples and providing fertilizers.

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