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

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**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 (ZnSO₄) 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 ZnSO₄ increased it. After six weeks, hatching was least (5.45%) in higher dose of urea and greatest (46.9%) in higher dose of ZnSO₄. In pot experiment, two doses of urea and single dose of SSP, MOP, and ZnSO₄ 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 ZnSO₄.

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**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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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 (ZnSO4) 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 J2 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.

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\text{% Larval emergence} = \frac{\text{Total larval emergence}}{\text{(Average cyst content} \times 10)} + \text{Total larval 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 J2/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 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 J2 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.

| Treatments | No. of hatched juveniles from 10 cysts | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | Total | Left over eggs cyst⁻¹ |
|------------|----------------------------------------|----------|----------|----------|----------|----------|----------|-------|----------------------|
| Urea 80 ppm | 0.00⁰| 8.75⁰| 26.00⁰| 57.00⁰| 35.25⁰| 40.25⁰| 167⁰| 221.75⁰|
| Urea 160 ppm | 0.00⁰| 4.25⁰| 19.50⁰| 45.00⁰| 32.25⁰| 35.50⁰| 136⁰| 234.75⁰|
| DAP 65 ppm | 0.00⁰| 23.25⁰| 44.25⁰| 79.50⁰| 47.00⁰| 53.50⁰| 247⁰| 216.5⁰|
| DAP 130 ppm | 0.00⁰| 17.25⁰| 37.50⁰| 71.25⁰| 43.25⁰| 48.25⁰| 217⁰| 220.5⁰|
| SSP 187 ppm | 0.00⁰| 37.75⁰| 59.00⁰| 86.00⁰| 59.00⁰| 69.25⁰| 31¹| 219.25⁰|
| SSP 375 ppm | 0.00⁰| 32.25⁰| 55.75⁰| 94.50⁰| 54.50⁰| 62.75⁰| 29⁰| 217.5⁰|
| MOP 50 ppm | 0.00⁰| 50.00⁰| 77.25⁰| 122.75⁰| 76.50⁰| 82.25⁰| 40⁰| 206.0⁰|
| MOP 100 ppm | 0.00⁰| 46.00⁰| 71.00⁰| 113.50⁰| 70.25⁰| 77.50⁰| 37⁰| 216.75⁰|
| ZnSO4 60 ppm | 3.75⁰| 99.25⁰| 156.50⁰| 208.00⁰| 126.25⁰| 141.75⁰| 73⁰| 113.25⁰|
| ZnSO4 120 ppm | 5.50⁰| 119.25⁰| 182.25⁰| 254.25⁰| 148.75⁰| 161.50⁰| 86⁰| 98.75⁰|
| Water (control) | 2.50⁰| 74.75⁰| 137.75⁰| 190.50⁰| 115.50⁰| 127.00⁰| 64⁰| 133.0⁰|

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 ≤ 0.05).

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 ZnSO4 and water. In second week, hatching was significantly less in all fertilizers than water, except in both doses of ZnSO4. Maximum hatching (119.25) was observed in ZnSO4 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, ZnSO4 stimulated hatching while other fertilizers inhibited it. Hatching was maximum (182.25) at higher dose of ZnSO4.

In all the treatments maximum hatching occurred in fourth week. At this time, maximum hatching was recorded in 120 ppm ZnSO4 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 ZnSO4. It is because urea suppressed hatching and more contents were left in the cysts. Contrary to this, due to stimulatory effect of ZnSO4, more larvae hatched and less contents were left in this treatment.

Hatching was significantly more in both concentrations of ZnSO4 as compared to control. At 120 and 60 ppm ZnSO4, 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.
3.2. Effect of fertilizers on hatching under pots

Data (Table 2) revealed that all the fertilizers treatment suppressed the hatching from cysts, except ZnSO₄. In all the fertilizers treatments, juveniles in soil were less than soil without fertilizers and this number was maximum in ZnSO₄ and lowest in higher dose of urea. ZnSO₄ 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 ZnSO₄. Average cyst content left in soil was affected by presence of fertilizers. It was lowest in ZnSO₄ and highest in urea at 326 kg/ha. In ZnSO₄, hatching was more than the combined application of all fertilizers but less than control. It is so because in combined application, stimulatory effect of ZnSO₄ 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 ZnSO₄ stimulated the hatching from cysts, except ZnSO₄. In all the fertilizers treatments, juveniles in soil were less than soil without fertilizers and this number was maximum in ZnSO₄ and lowest in higher dose of urea. ZnSO₄ 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 ZnSO₄. Average cyst content left in soil was affected by presence of fertilizers. It was lowest in ZnSO₄ and highest in urea at 326 kg/ha. In ZnSO₄, hatching was more than the combined application of all fertilizers but less than control. It is so because in combined application, stimulatory effect of ZnSO₄ 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.

![Fig. 1. In vitro effect of fertilizers on hatching of Heterodera avenae.](image-url)
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 NH₄Cl, (NH₄)₂SO₄ and NH₄NO₃ 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 ZnSO₄ 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* 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 stimulating effect of the fertilizers can be directly beneficial to crop for protection against nematode. Hatching stimulating effect of ZnSO₄ 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.

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