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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON LIFE-CYCLE OF *HETERODERA AVENAE* INFECTING WHEAT

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Abstract – *Heterodera avenae* commonly known as Cereal Cyst nematode causing 'Molya' disease of wheat and barley dominates in Rajasthan. Arbuscular Mycorrhizal Fungi (AMF) associations with plants are geographically ubiquitous and their eco-friendly role in enhancing resistance in plants is well appreciated, widely studied and reported. Hence, present study was conducted in order to study its effect on the lifecycle of *Heterodera avenae* on wheat. It was found that AMF delayed *H.avenae* penetration and development, reduced the number and viability of eggs, and decreased the morphometric dimensions and life-cycle duration in mycorrhizal inoculated roots as compared to non-mycorrhizal roots. Better plant growth was observed in *Glomus fasiculatum* treated plants as compared to untreated plants.

INTRODUCTION

The most challenging task in sustainable agriculture at present is the efficient control of pests including wide variety of soil pathogenic nematodes infecting economically important crops and cereals. The extent of loss caused by these Plant parasitic nematodes in agriculture is about 20.6% losses in terms of total biomass production and yield as reported by many researchers (Jain *et al.,* 2007). However due to the non judicial use of the synthetic and chemical pesticides, fertilizers and resistant crop varieties to manage plant-parasitic nematodes has lead to degradation and pollutionof our biosphere, contamination of the environment and development of resistance in pathogens. It has created worldwide interest in biological pest control agents like bio-pesticides, organic manures, oil-seed cakes, chopped plant parts and AMF which are eco-friendly, easily available, economical and biodegradable.

Arbuscular mycorrhizal associations - a common form of symbiosis between plants and fungi are geographically ubiquitous (Sharma and Batra, 2014). In past few decades, discoveries by scientists on AMF have generated an explosion of interest in the field of mycorrhizal research (Chawla *et al.,* 2009; Sharma and Yadav, 2013). The interaction between AMF and plant parasitic nematodes has been studied and the reduction in nematode population has been reported by many workers (Sharma and Trivedi, 2001; Arya and Saxena, 2009). Cyst nematode, *Heterodera avenae* dominates in the wheat growing fields of Rajasthan state, India causing 'Molya' disease of wheat. In the regional language 'Molya' means deformed and decreases wheat production which is one of the major cereal crops of Rajasthan state. Thus checking this disease on the right time is essential which required the basic fundamental knowledge about various important aspects such as biology, host range, host parasite relationship and many more. The biology of the pest portrays various developmental stages whichoccur in the life cycle. This helps us in selecting and evaluating a particular stage of life-cycle of pest, at which proper control or management measures could be applied and further disease multiplication could be checked. A preliminary survey was done to study AMF association in rhizosphere of wheat and it was found that AMF prevailed in good % in most of the wheat cultivated fields with *G. fasiculatum* as dominant species. Hence, present study was undertaken with an aim to study the effect of AMF on the lifecycle pattern of *H. avenae* infecting wheat

MATERIALS AND METHODS

Life-cycle of H. avenae: For the present study fifteen local wheat fields were surveyed and soil samples were collected and processed to determine the AMF presence and *H. avenae* cysts. The lifecycle was studied in 10 cm plastic pots. Seeds of wheat variety WH-147 were used for experimental purpose. The seeds were surface sterilized by 0.1% $HgCl_2$ for 2 minutes, washed with distilled water and then sown in 15cm earthen pots containing autoclaved soil. To provide nutrients to plants around Five ml of Hoagland's complete nutrient solution, once a week was added to pots under study. Since wheat is a rabi crop all experiments were carried out between november to march. Azygospores, chlamydospores, infective fungal hyphae and fungal roots all comprised mycorrhizal inoculums which were inoculated just below the wheat seeds during sowing. Two different sets including mycorrhizal and non mycorrhizal experiments were replicated four times.

Preparation of H. avenae inoculum: Extraction of *H. avenae* cyst from native soil was done by simple flotation and visual screening process. Soil containing cysts were placed 80-mesh sieve of 175u pore size and washed through running water. Residue left was washed, filtered and examined under microscope. Cysts were picked and collected in vials. Cysts were also extracted from roots of infected roots at the time of harvesting.

Hatcting of cysts and penetration: The collected healthy cysts were crushed in distilled water as to obtain second stage juveniles for inoculation. The resulting suspension with eggs was incubated in BOD at optimum temperature 18±1^{0}C for 48 hours measured by counting their number present per ml of suspension under microscope. For nematode counting 2mL of the resulting suspension were pipette out in the multi-chambered nematode counting dish and the counting was done under microscope. Mean of five such readings were taken and finally the total number of juveniles for inoculation was calculated. Nematization or second stage juvenile inoculation was done by pouring larval suspension @1000 larvae per pot. The pots were arranged in randomized complete block design.

Life-cycle of H. avenae: Observations were made after every 24 hours till the recovery of 2nd generation. After inoculation the seedlings were uprooted daily for first seven days, at three days interval till fifteen days and thereafter weekly till ninety days. The number of days of moulting of various developmental stages of juveniles up to adult was noted. The roots obtained were washed in water, stained with 0.1% acid fuchsin in lactophenol for1-2 minutes and studied under microscope (McBeth *et al.,* 1941).

RESULTS

The life-cycle of *H .avenae* consisted of various stages from hatching of eggs, second stage juveniles, penetration, third stage juvenile, fourth stage males and females, adults, cysts to again second stage juveniles. After first moulting, second stage juveniles with a well developed stylet emerged from eggs which were very active. The second stage juveniles were vermiform measuring 446-590 x 38-54 um in dimension (Fig. 1). The penetration rate

Table 1. Comparative life cycle duration of *Heterodera avenae* on wheat as influenced by the application of AMF

S. No.	Penetration and Development of *Heteroderaavenae* on wheat	AMF treated wheat plants	Untreated wheat plants
1.	% Penetration	40-45%	50-55%
2.	Penetration Time	Within 72 hours	Within 48 hours
3.	Moulting second stage juveniles	6	5
4.	Early third stage	13-14	11-12
5.	Third stage male	17	15
6.	Third stage female	21	18
7.	Fourth stage male	25	21
8.	Fourth stage female	30	27
9.	Adult male	31	28
10.	Adult female/white cyst	48	42
11.	Yellow cyst	59	54
12.	Brown cyst	68	62

and the development of larvae in the infected cysts of wheat root seem to be effected. The freshly hatched active second stage juveniles penetrated roots of non-mycorrhizal plants within 48hours whereas in mycorrhizal the penetration was delayed till 72 hours. The percent penetration was less in AMF treated roots. As a result many endodermal and cortical cells were ruptured.

The second stage larvae entered the third stage larvae from 13-14th and 11-12th days after nematode inoculation in *Glomus fasiculatum* treated and untreated plants respectively (Table 1). Changes in the body dimensions of the third stage larvae were observed after their establishment in the stelar region and the larvae became stouter and thicker with time (Table 2).

DISCUSSION

Overall results unveiled that penetration time by *H. avenae* was delayed in *G. fasiculatum* treated plants as compared to non-mycorrhizal plants. This can be attributed to heavy AMF colonization in wheat roots which in turn causes hindrance in juvenile penetration. In this case total number of cyst at harvesting was more in non-mycorrhizal roots as compared to to *G. fasiculatum* treated roots. Similar results were reported by Sharma and Swarup (1988) on *H. avenae*. In addition to the reduced rate of nematode penetration in the roots of AMF plants, Price *et al.*, (1983) also found specific decrease in the reproduction rate of parasite.

The present study divulged that *H. avenae* completed its elopement from second stage juvenile to white cyst in lesser days than untreated wheat plants. This delay could be due to delay in penetration time or due to less attraction between host root and second stage juveniles, both of which were influenced by AMF application (Sharma and Yadav, 2013). Not only the number of larvae penetrating the host root was decreased due to reduced egg and larvae in the AMF influenced cysts but the rate of development of the penetrated larvae was also retarded. There was a decrease in the reproduction rate also. This is in collaboration with findings by Dehne (1982) who stated that AMF are competitors of sedentary nematodes.

It was recorded in *G. fasiculatum* inoculated plants that not only disease incidence was reduced but enhancement in plant growth and vigour was observed. Reduction in severity of cyst number in mycorrhizal plants was may be due to improved plant nutrition especially phosphorus and improved plant growth was due to increased area of surface absorption in mycorrhizal roots (Jalali and Chand, 1990; A1-Nahid and Gomah, 1991). Morphometric measurements and time duration of different larval moultings was observed and it was seen that the duration for the fourth stage male larvae was very short and it was difficult to mark the end of this

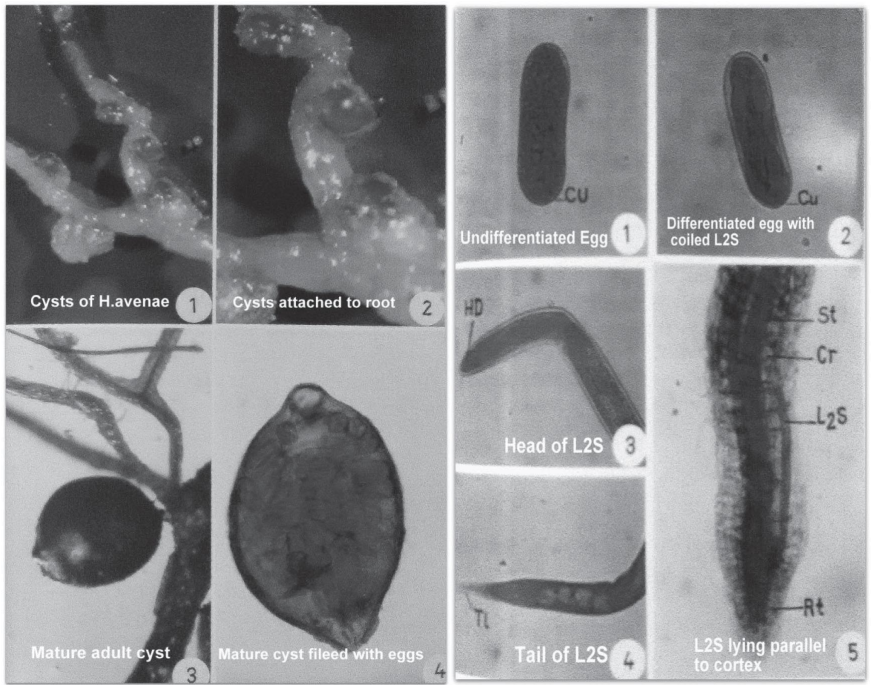

Fig. 1. Life-cycle stages of Cereal cyst nematode – *Heterodera avenae* infecting wheat.

Table 2. Morphometrics of the different developmental stages of *Heterodera avenae* in AMF treated and untreated plants of infected wheat (mean of five replicates).

S. No.	*H. avenae* developmental stages	Range in AMF treated plants L x W (um)	Mean(um)	Range in Untreated plants L x W (um)	Mean(um)
1.	2nd stage Juveniles	431-560 x 36.5-50.8	495.5 x 43.65	446-590 x 39.5-54.8	518 x 47.15
2.	Early 3rd Stage Juveniles	442-515.2 x 96.5-100.8	478.6 x 98.65	451-512.5 x 99.5-118	481.75 x 108.7
3.	Late 3rd stage males	450.5–468 x 91-115	459.1 x 103	461.7-480.5 x 99.5 -118	471.1 x 108.7
4.	Late 3rd stage females	567.5-610.2 x 148 - 240.5	588.85 x 194.25	586.2-630.5 x 143-230.6	608.3 x 197.2
5.	4th stage males	471.8-506 x 85-96.3	488.9 x 90.65	489-516.5 x 96-112	502.7 x 104
6.	4th stage females	515.2-640 x 116-211.2	577.6 x163.6	535.8-681.5 x 121-273.5	608.6 x 197.2
7.	Adult male	1380.5-1487.2 x 30.5-46.2	1433.7 x 38.35	1439-1561 x 36.5-49	1500 x 42.75
8.	Adult female	630.5-681 x 515-589.8	655.7 x 552.4715.5-763	715.5-763 x 590-630.2	739.25 x 610.1
9.	Egg	81.2-107 x 38-41.5	94.1 x 39.75	103.5-116 x 45.2-47	109.7 x 46.1

stage. The 2nd stage Juveniles, Early 3rd Stage Juveniles, Late 3rd and 4th stage males and females, adult male and females and eggs in non mycorrhizal wheat roots portrayed better size and shape as compared to mycorrhizal infected wheat roots. Taya and Bajaj (1986) studied larval stages of different *Heterodera* species and found similar results. The wheat roots greatly colonized with mycorrhizal fungi are found competent to grow sound instead of existence of damaging levels of nematodes and in addition AMF has been reported to augment overall vigor and growth of a variety of crop and vegetable plants (Thomas *et al.*, 2000).

CONCLUSION

The attraction, penetration and developmental studies of *H. avenae* were carried on wheat variety WH-147, in myco and non-mycorrhizal roots and were observed after definite intervals. Based on the present results it can be said that not very much difference was observed in the morphometrics and different developmental stages from $Juvenile_2$S to white cyst in mycorrhizal and non mycorrhizal wheat plants through penetration time and percent penetration was decreased in AMF inoculated plants which indicates its considerable utility in controlling disease incidence. Percent penetration and development time in *G. fasiculatum* treated wheat plants was quite more due to its protective mechanism and enormous potential to reduce *H. avenae* population in contrast to untreated wheat plant. Overall it can be concluded that in AMF inoculated plants the life cycle of pathogen was adversely effected and there was delay in all the life processes starting from penetration to moulting of larvae, their development and maturation of male and female adults to finally the mature cyst. The morphometric data also showed similar trend in body dimensions that is less number of viable cysts were present in plants with AMF infection. Thus application of AMF fungi is an economic, prospective and effective tool for reducing *H. avenae* population causing significant crop losses.

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