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Survival and infectivity of *Meloidogyne javanica* juvenile under extreme condition

Sheikh Sonia Aurmy[1], Md. Rasal-Monir[2]*, Sourav Modak[2], Sumya Fatima[3], Md. Rubel Mahmud[1] and Shah Md. Asraful Islam[1]

[1]Department of Plant Pathology, Patuakhali Science and Technology University, Dumki, Bangladesh.
[2]Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.
[3]Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

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Corresponding Author

Md. Rasal-Monir
Email: monirr718@gmail.com

ABSTRACT

A experiment was carried out to estimate survival, hatching and infectivity of *Meloidogyne javanica* juvenile at extreme condition. The effect of different temperature regimes (4, 25 and 35°C) up to 4 weeks and desiccated condition of J2 (juveniles) up to 60 minutes on the hatching of eggs, survival and infectivity of eggs and J2 were examined by a series of experiment. Results showed that temperature was the most important factor for nematode survival. Highest temperature (35°C) dramatically altered the parameters of interest. At very low (4°C) and high (35°C) temperature more than 70% of eggs were survived and hatched within the 1st week in water followed by gradually decreased with the increasing of exposure time. At very low temperature a better number of eggs were hatched even after 4th weeks (48.54%) compare to high temperature (5.36%). At 25°C, more than 80% eggs were hatched up to 2 weeks then gradually decline up to 4 weeks. In desiccation experiment, survival of J2 was found higher at 25°C up to 60 minutes. On the other hand very low survival was observed at 35°C after 60 minutes exposure of J2. At 4°C some juveniles can survive but a bit higher survival rate was observed than 35°C temperature up to 60 minutes. When the treated J2 were infected in brinjal plant, the highest gall (28 galls/plant) was recorded at 25°C. The lowest gall (2 galls/plant) was formed from 4°C treated juveniles. So, treated with higher temperature (35°C), most of the juvenile were paralyzed and did not penetrate into the root tissue. At 4°C the J2 can go for dormant condition and when favors, infection occurred in root tissue.

Introduction

Root-knot nematodes are included within the genus *Meloidogyne* and one of the most polyphagous and ubiquitous genera of plant-parasitic nematodes. These nematodes infect thousands of different herbaceous and woody monocotyledonous and dicotyledonous plants and cause serious losses to numerous agricultural crops worldwide (*Eisenback and Triantaphyllou,* 1991). Presently, 98 species were described in the genus Meloidogyne.

Meloidogyne javanica is a species of nematodes (roundworms) in the family Heteroderidae. It is commonly called the 'Southern root-knot nematode.This parasitic roundworm has worldwide distribution and numerous hosts.It is an important plant parasite classified in parasitology as a root-knot nematode,as it prefers to attack the root of its host plant.When *Meloidogyne javanica* attacks the roots of plants, it sets up a feeding location, where it deforms;the normal root cellsand establishes giant cells.The roots become gnarled or nodulated (knobbly,rough and twisted), forming galls,hence the term 'root-knotnematode. Meloidogyne javanica has been found to be able to move along shallower temperature gradients (0.001C/cm) than any other known organism an example of thermo taxis.

Nematodes are a highly remarkable group of organisms that exhibit different adaptations in adverse soil and plant environments and to withstand host response (Perry, 2012). The first description of a plant-parasitic nematode was Anguina tritici (Needham, 1743) which was also the first description of a nematode in a state of crypto or anabiosis. Meloidogyne spp. are soil dwelling aquatic animals that are dependent on external sources of heat and water for their development, hatch and movement.

In addition, since they are obligate plant parasites, the infective juveniles have limited stored reserves for survival in the absence of a host and the survival duration is determined by temperature and moisture.While much is known about environmental influences on the life cycle of Meloidogyne spp. The influence of soil temperature and moisture on *Meloidogyne javanica* (Kofoid and White) was examined in relation to hatching and survival of second-stage juveniles (J2).

Nematode life cycle and behavior, including hatching, motility, invasion and development are influenced by temperature. Survival strategies of root-knot nematodes are dependent on abiotic and biotic conditions. To escape from extreme environments, some species may undergo a dormant stage and postpone their development resulting in difficult control Das, 2011). A number of eggs in egg masses from *Meloidogyne javanica* ceased their development and went to a resting stage even when the environmental conditions were favorable (de Guiran, 1979). Very limited work is available on the study of temperature and

desiccation survival and hatching of J2 of *Meloidogyne javanica*. Eggs within the egg mass will be exposed to different temperatures and desiccated environments to analyze the survival of eggs and infection capability of J2 under different conditions.However,temperature, desiccation and host plant have influence on the survival of eggs in gelatinous matrix of *Meloidogyne javanica*. Present investigation is innovative approaches to evaluate the survival *of Meloidogyne javanica* eggs and juvenile under extreme conditions (35^{o}C and 4^{o}C) temperature in gelatinous matrix and to investigate the infectivity of the J2 for survival in the brinjal plant.

Materials and Methods

Experimental site and experimental period

The present investigation was carried out during July 2017 to December 2017 in the Plant Disease Clinic (PDC) and Plant Pathology Laboratory under the Department of Plant Pathology, Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali

Environment of experiments

Experimental plants were kept in the house where the temperature was 30 ± 2o C during the “day” and 23 ± 2o C during “night” with an average temperature of 28± 2o C.

Pot Experiment

Brinjal cv Singnath was used for the experiment. It is an important, popular, cheap and common vegetable in Bangladesh.

Collection of seeds

Healthy, mature and disease free seeds of singnath variety were collected from the local market of Dumki upazilla of Patuakhali district.

Soil collection and sterilization

Required soil sand and decomposed cow dung were collected from Field laboratory of Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali. Then soil (sand 80%, loam 10%, clay 10%) and cow dung were mixed properly. The mixture was autoclaved at 121ºC for 15 minutes at 15 PSI. The sterilized soil was allowed to room temperature followed by filled in the plastic trays for raising seedlings.

Raising of seedlings

Plastic trays were filled with sterilized and fertile soil. Seeds of brinjal were soaked in sterile water for better germination. Then the seeds were sownin the plastic tray. The trays were covered with polyethylene sheet and kept in sunlight for raising seedlings. Seedlings were observed regularly and watering was done as per necessity up to transplanting in poly bag.

Preparations of pots

Plastic pots of 1500 cm3 were cleaned, washed, dried up properly and sterilized with 70% ethanol properly. Sterilized and fertile soil was filled in required amount into each pot. Each pot contains 1.5 kg soil. Then the pots were arranged according to experimental design.

Inoculation of Meloidogyne javanica

J2 of nematode (*Meloidogyne javanica*) was collected from severely galled roots of brinjal. Juveniles (J2) were extracted through Baerman funnel technique. The number of eggs per egg mass was counted. Each plant was inoculated with 1000 J2 next day of transplanting, by using micropipette making three holes surrounding the transplanted seedlings.

Nematode culture

One root-knot nematode (Meloidogyne javanica) cultures was maintained on susceptible brinjal plants (cv. Singnath) under controlled conditions at PLP field laboratory, PSTU. Plastic pots (17 cm diam) were filled with sterilized soil (87% sand, loam 9% and clay 4%). Then a single brinjal seedling (4-leaf stage) was transplanted to each pot. Each pot was inoculated by approximately 2000 freshly hatched J2 of each species. The initial inoculum was collected from pre stock culture of PDC lab PSTU.

Collection of egg masses

Egg masses were collected from 12-15 week old brinjal plants. The brinjal roots were washed with water to remove the adhering soil. Then small pieces of root (about 5 mm in length) cut containing eggs with gelatinous matrix for hatching tests. Egg masses were obtained by agitating root pieces (1.0-2.0 cm long) rinsing with tap water over 60 and 30 micro meter sieve. The egg masses were transferred into a 250 ml beaker to get egg suspension.

Baermann funnel technique

Nematodes from roots were extracted by modified Baermann funnel technique to the funnel stem and (Baermann, 1917). In this technique, a funnel was taken in which a piece of rubber tube was attached the rubber tube was closed with a pinch clamp (clip). The funnel with this arrangement was supported on a ring of a stand.

A nylon net attached to a ring was placed on the funnel. Water was poured on the funnel till the water surface reached to a maximum upper level of the funnel and touched the nylon net Roots were chopped into small 1 cm pieces and l0 g of chopped roots were sprinkled over the nylon net on the funnel so that counting dish by carefully roots were just submerged. Nematodes emerged from the

tissues and sank to the bottom of the funnel stem at the clip level. After 24 hours, a small quantity (10ml) of water was taken in a opening the clip of the attached rubber tube. Nematodes were counted under sterio microscope. Each process was replicated thrice.

Survival Experiment

There, two types of experiment were carried out to show the Survival of Meloidogyne javanica juvenile under extreme condition.

Plate.1: Nematode suspension (J2) was inoculated by digging holes

Influence of temperature on survival of eggs in gelatinous matrix and the infectivity of the hatched J2 of Meloidogyne javanica into the brinjal plant. 35°C). Viability of eggs and hatched juveniles of egg masses assessed under astereo microscope.

Plate.2: Baermann funnel Method

Egg masses were harvested and placed in a glass petridish. Then petridishes were transferred into a desiccation box with silica gel in incubator. Hatched J2 and unhatched eggs from egg masses were counted followed by performance of the infectivity test on juveniles from eggs.

Infectivity test

The relative infectivity of J2 that hatched immediately under extreme conditions (Desiccation and extreme temperature) from both conditions of eggs determined by infecting egg plants. Brinjal plants were grown in sterilized soil (87% sand, loam 9% and clay 4%) in plastic pots (dia 7 cm and 10 cm deep). Five holes were made in their bases to inoculate J2 (about 250) to infect the plant and check the penetration after one week with fuchsin acid staining.

Data analysis

All data collected on different parameters were analyzed following statistical method using SPSS. Hatching percentage and infectivity test will be calculated for all replication of treatment by ANOVA. Mean comparison can be done by Tukey test

Results and Discussion

Growth Characteristics

Plant survivability

The interaction effects were found insignificant on plant survivability (Table 1). The higher percentage of survivability might be the consequence of proper care and management.

Plant Vigor

Plant vigor varied significantly due to the interaction effect of planting time and densities of top shoot cuttings (Table 1). The plants of earliest plantings with wider spacings (P_1S_3) showed maximum vigor (8.00) which was statistically similar with P_1S_2 (7.33) followed by P_1S_1 (6.67), P_2S_3 (6.33). The lowest vigor (3.33) was obtained from P_3S_1 and P_3S_2.

Table1: Survival (%) of *Meloidogyne javanica* (eggs) at different temperature and exposure time in water.

Temperature (°C)	Time(weeks after hatching) 1st	2nd	3rd	4th
4	$^{B}70.08_{a}$	$^{B}62.53_{a}$	$^{B}56.26_{b}$	$^{B}48.54_{bc}$
25	$^{A}85.73_{a}$	$^{A}82.50_{a}$	$^{A}72.28_{b}$	$^{A}61.26_{c}$
35	$^{B}78.54_{a}$	$^{C}50.60_{b}$	$^{C}30.26c$	$^{C}5.36_{d}$

Means with the same letter superscripts do not differ significantly at 0.05 level using Tukey test. The upper case superscripts represent the vertical comparison of the survival of root-knot-nematodes (J2) over different temperatures at certain time. The lower case superscripts represent the horizontal comparison of the survival rate of *Meloidogyne javanica* over different weeks at certain temperature.

At 4°C temperature significantly different in hatching from eggs masses of *Meloidogyne javanica* were observed at the different observationtime.Hatching was gradually decreased with the increasing of exposure time.The highest hatching was observed at 1st week 70.08% followed by 2nd week 62.53% almost same, but at 3rd week hatching was 56.26%.The lowest hatching rate was observed at 4th week later 48.54%.

At 25°C temperature the highest hatching was observed at 1st week (85.73%) followed by 2nd week (82.50%). Hatching was significantly reduced after 3rd weeks (72.28%) and on words. The lowest hatching rate was observed at 4th weeks (61.26%).
At 35°C temperature significantly different in hatching of Meloidogyne javanica fromwere observed at the differenttime points.Hatching was gradually decreased with the increasing of exposure time. The highest hatching was observed at 1st week 78.54% and 2nd week was 50.60%. At 3rd week hatching was 30.26%.The lowest hatching rate was observed at 4th week later 5.36%.

There was significant variation was observed among the temperatures (4, 25 and 35 °C). After 1st week the highest hatching was observed at temperature 25°C (85.73%) and lowest at both 4°C (70.08%) and 35 °C (78.54%). From the 2nd week on words, the highest hatching was observed at 25°C, and lowest at 35°C. In 2nd week the highest hatching was observed at 25 °C (82.50%) then 62.53% at 4 °C and 50.60% at 35 °C.After 3rd week the highest hatching was observed at the same temperature 25°C (72.28%) and at 4°C (56.26%) and 35°C (30.26%). Sothat 4th week the hatching was observed at the lowest rate. Among them the highest was in 25 °C (61.26%) followed by 48.54% at 4 °C. The lowest rate was observed 5.36 % at 35 °C.

Desiccation on survival of second-stage juveniles of Meloidogyne javanica in different temperature

Effect of desiccation on survival of second-stage juveniles of M. javanica at 4°C temperature.

At 4 °C, 28% J2 was found survivewithin 5 minutes of exposure, followed bymsharply decreased of J2 survival over time. After 60 min of exposure, 10% of J2 was found survive.

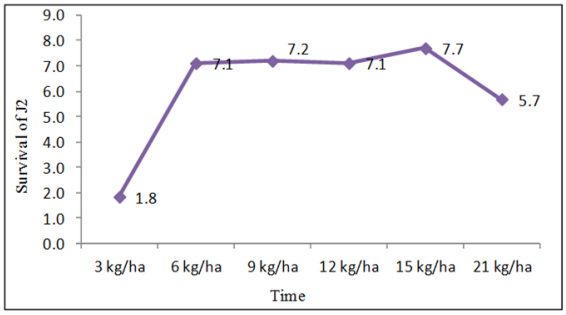

Fig.1 The survival of Meloidogyne javanica after various periods of exposure at 4°C temperature.

Effect of desiccation on survival of second-stage juveniles of M. javanica at 25°C temperature.

Survival rate more or less similar (48%) up to 60 minutes exposure of J2 at 25°C

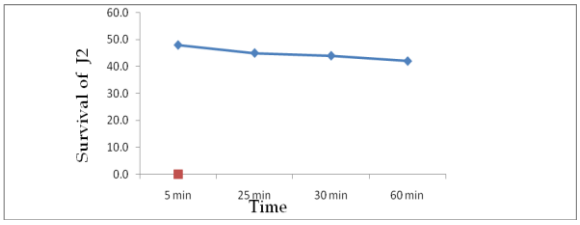

Fig.2The survival ofJ2 of Meloidogyne javanica after various periods of exposure at 25°C temperature.

Effect of desiccation on survival of second-stage juveniles of M. javanica at 35°C temperature

38% J2 of Meloidogyne javanica was found survive up to 5 minutes exposure at 35 °C and lowest (2%) survival of J2 was observed after 60 minutes exposure at 35°C. Survival rate was gradually decreased with increasing of exposure time.

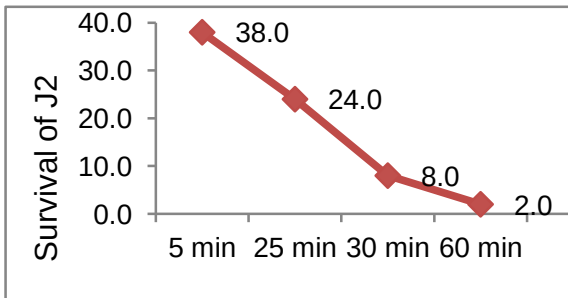

Fig.3: The survival of J2 of Meloidogyne javanica after various periods of exposure at 35°C temperature.

So, here J2 survival rate was gradually decreased with increasing of exposure time in desiccation experiment.

Infectivity test

Among three temperatures, at 25°C treated J2 were highest infective after penetration of J2 into the brinjal plant, 28 galls/plant was observed at 25°C temperature. At 4°C treated J2, 12galls/plant was recorded. The lowest gall (2galls/plant) was observed at 35°C treated J2.

Table:2 Effect of treated temperature on infectivity of second stage of Meloidogyne javanica (J2) into the brinjal plant.

J2 treated temperature (°C)	No. of galls/plants
4	12
25	28
35	2

The infectivity was decreased with increasing temperature up to 35°C.But at normal temperature 25°C the rate of infection was highest.

The development (Wallace, 1966) and hatching (Bird & Wallace, 1965) of nematodes in the genus Meloidogyne dependent on external sources of heat and water. In this experiment, survival and hatching of Meloidogyne javanica was depending on temperature and time. In an in vivoexperiment, (Towson & Apt 1983) found that the duration of the survival of Meloidogyne javanica is determined by temperature and time exposure .

The present study was carried out with survival of eggs, juvenile of Meloidogyne javanica in different temperature. Further it is investigated the infectivity into the brinjal plant by treated J2 of Meloidogyne javanica. Previous investigatorsmentioned that the J2 of root-knot nematodes in the soil are vulnerable to environmental stresses(Moens et al., 2009).More than 70% of the Meloidogyne javanica remarkably survived when subjected to 4°C in the 1stweek while more than 60% survived up to 2nd week after hatching. The highest hatching rate was observed at 25°C in 1st week (85.73%) followed by 2nd week (82.50%) almost statistically similar, but at 3rd week hatching was 72.28%. Hatching was gradually decreased with the increase of temperature (35°C) and exposure time. The lowest hatching rate was observed in 4th week later (5.36%) at 35°C. It is reported earlier that 10 weeks after hatching at 4°C more than 60% of M. javanica survived, while more than 80% of M. javanica survived at 25°C until 2nd weeks but the survival rate is decreased at 3rd and 4th weeks at the same temperature (Das, 2010).So the temperature 25°C is more favorable for hatching, survival and infection of Meloidogyne javanica. At low temperature (4°C), the egg masses and J2 can survive in dormant with water and soil. When the temperature being favorable they become infective and infect the root tip of host plants (egg plants) and forming galls.

Previous scientists also mentioned temperature among a host of factors that can limit or stop the movement of root-knot nematodes through the soil (Curtis et al. 2009 and Moens et al. 2009). In this experiment, high temperature at 35°C showed relatively high impact on the survival of the tested nematodes. In the case of *M. javanica* a poor survival proportion of less than 50% was recorded from the 2ndweek onwards to reach 5.36% at 4th week.Temperature showed an inverse relationship with infectivity for M. javanica up to 4thweek after hatching, at both low and high temperatures. When J2 was treated in temperature 25°C but lower at higher temperature 35°C.Similarly, Rocha et al. (2009) reported that high temperatures resulted to the rapid loss of infectivity of Meloidogyne J2. Infectivity of M. javanica J2 declined from 63% when stored at 25°C for 16 days (Van Gundy et al., 1967) or up to 98% when stored at 28 °C for 6 days (Freire et al., 2007).

It was reported that the optimum temperature for hatching Meloidogyne species was 20°C, but J2 of M. javanica hatched more between 20 and 25°C(Khan et al., 2014).At low temperature a number of unhatched eggs went to either a state of quiescence or diapauses. In the light of different findings, including the present study it can be said that gelatinous matrix have a protective role for the survival of eggs inside under adverse environmental conditions for long period of time.

So these findings not only provided information on the biology and physiology of the nematodes but also basic information for nematode control strategies, such as solarization, ploughing, irrigation and fallow practices. Solarization has been used for nematode control. The present study showed, at temperature 35°C or very low temperature 4°C, a substantial number of eggs were unhatched in egg masses and goes to either quiescence or diapauses. At very low temperature developmental stages inside the egg become arrested. Upon return in favorable conditions, further development and activity started inside the eggs to result in hatching of the J2. A significant amount of hatch occurred from egg masses of both moist and dry soil but gradually declined up to 4 weeks. The decline rate was higher in high temperature (35°C). So, higher temperature or longer exposure was needed to kill the eggs with the protection of the gelatinous matrix.

Conclusion

This experiment was conducted to evaluate the survival, hatching and infectivity of second-stage juveniles of *Meloidogyne javanica* under 3 different temperatures (4, 25 and 35 °C). High and low temperature gave better response in suppressing the development of egg masses and juveniles (J2). The study showed that more than 70% of the juveniles remarkably survived when subjected to 4°C in the 1st week while more than 60% survived up to the 2nd week after hatching while more than 80% of M. javanica survived at 25°Ctill 2nd weeks but the survival rate was decreased at 3rd and 4th weeks at the same temperature. On the other hand,

high temperature (35°C) showed relatively high impact on the survival of the tested nematodes. So the experiment explored the possibility of using different temperature and desiccated condition to reduce infectivity and survival of root-not-nematode and this is eco-friendly management of root-knot of eggplant caused by *Meloidogyne javanica*.

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