# IMPACT OF INDUCED STRESS ON PARASITIC AND FREE LIVING NEMATODES FROM SELECTED AREAS OF GUJARAT: A PRELIMINARY STEP AIMED AT NEMATODE CONTROL

ZANKHANA R. PANDIT*, HYACINTH N. HIGHLAND, LINZ-BUOY GEORGE

Nematodes are a diverse group of soil fauna, the group of roundworms belonging to the phylum Nematoda. More than 14,000 nematode species have been described, distributed in almost every habitat, from free-living forms to parasitic types. In the present study, we identified five nematode species from Fenugreek leaves (*Trigonella foenum-graecum* L.), Green onion (*Allium fistulosum* L.) and Ginger (*Zingiber officinale* Roscoe), from freshwater bodies, as well as from neighbouring privately owned farmlands in the Village Boriavi, District Anand, Gujarat. After identification and classification nematodes were cultivated on Nematode Growth Medium (NGM) plates and assays for various stress parameters such as salinity, heat and pH were carried out to evaluate the influence of altered physical conditions on growth and survival of these organisms. The data obtained would facilitate measures for the control of agricultural pest nematodes. Identification studies revealed the presence of *Prodontorhabditis* spp. from Green onion (*Allium fistulosum*), *Eudorylaimus* spp. from Ginger (*Zingiber officinale*), *Doryllium minor* and *Radopholus* spp. from root soil of *Trigonella foenum-graecum* L. Anand district and *Aporcelaimus* spp. from freshwater bodies, Ahmedabad. Salinity stress was observed to increase the mortality rate in *Prodontorhabiditis* spp., whereas, specified increased salt concentration appeared to have growth promoting effects in juveniles of *Doryllium minor* and *Radopholus* spp. The results of the heat stress assay indicated that temperatures above 40ºC prove lethal for all test nematode species, with fatal effects in all developing stages. The results of the pH stress assay suggested that plant parasitic nematode species showed optimum growth at pH of 8 for *Radopholus* spp. Hence, maintaining pH conditions of pH 9 would prove effective in controlling the plant parasitic nematodes i.e. *Radopholus* spp. and this pH level would not adversely affect free living nematode species i.e. *Prodontorhabditis* spp., since they showed favourable growth conditions at a pH range from 8.5 to 9.0. Thus, the study helped to identify crucial stress conditions, which could help curtail growth of parasitic nematodes by altering the ambient soil environment.

*Keywords:* Salinity Stress, pH Stress, *Radopholus* spp., *Prodontorhabditis* spp., Heat stress.

## INTRODUCTION

Nematodes have successfully adapted to nearly every ecosystem from marine to freshwater, in varied soils, from polar to tropic regions, as well as parasites of animals and plants. Plant parasitic and free living nematodes are known to tolerate a wide range of environmental disturbances and are recognized to adapt quickly,

a phenomenon that aids in the evaluation of environmental health. Nematodes are useful indicators of environmental quality and have been reported to show sensitivity to a wide range of stress factors such as heat, salinity, temperature and pH (Dong *et al*., 2018). Stress assays are generally carried out using the free living, marine nematode *Caenorhabditis elegans,* as it is a research model species and a representative of phylum Nematoda. Consequently, *C. elegans* survival assays have proven to be key tools for studying stress response and physiological processes including aging, stress resistance and immunity. A remarkable advantage of using *C. elegans* for such assays is that synchronized isogenic populations are simple to obtain since the worm usually exists as a self-fertilizing, hermaphrodite that produces hundreds of isogenic progeny (Park *et al*., 2017). However, stress assays on plant parasitic nematodes are rarely done although such assays are inevitable, as these parasites have a major impact on agricultural yield, quality of food, as well as on the economy of agricultural outcome.

Stress assays mainly disrupt protein homeostasis (e.g., Heat stress assay) and in addition, adversely affect physiological activity, certain morphological features and behavioural patterns. Hence, evaluation of stress induced alterations and a study of the outcome of stress assays would provide valuable information for controlling plant parasitic nematodes in agriculture. It is thus, an environmentally friendly mode for sustainable agricultural development.

Many nematodes are well adapted to abiotic stress and are capable of cryptobiosis (hidden life): the ability to enter a state of suspended metabolic activity during unfavourable environmental conditions (drying, heat and cold). While not all nematodes are capable of cryptobiosis, the ones that are can often survive for years in a cryptobiotic state awaiting favourable conditions that will trigger their revival. The ability of nematodes to undergo cryptobiosis and overcome stress is one reason why some nematode species are very difficult to control or eradicate from a field (Kumar & Yadav, 2020). Some nematode species like *C. elegans* activate a specific and unique stress response when subjected to a combination of multiple abiotic and biotic stresses. Plant parasitic nematodes have been found to reduce stress tolerance levels in plants which lead to quality loss and yield loss of crop in agricultural fields (Atkinson & Urwin, 2012).

The influence of stressors on soil-borne pathogens has been a neglected area in stress research as most studies have been directed towards understanding stress response in plant parasitic nematodes (PPN) since they contribute to vast agricultural losses (Eastburn *et al*., 2010). Infestation of crops with plant-parasitic nematodes can exacerbate or counteract the effects of abiotic stress on plants, as their parasitic attack on roots severely disrupts plant water absorption (Bird, 1974; Smit & Vamerali, 1998). Very few sporadic research reports are available regarding the physiological stress responses of soil or plant parasitic nematodes in the State of Gujarat and hence there is now an urgent need to investigate altered stress conditions in nematodes of this region to provide insights for their control and suppression. Monitoring, curtailing and eradication of nematode attack are imperative to create avenues for developing healthy plants that maintain high yields.

The aim of this study was to evaluate the impact of induced stress on certain selected plant parasitic and free living nematodes collected from agricultural fields, using standard stress assays. The results obtained after carrying out the selected stress assays are beneficial for standardization of specific plant parasitic nematode cultivation methods under laboratory conditions. In addition, the data obtained following stress assays would help to reveal sensitivity of nematode species to altered environmental conditions and this in turn would prove useful in designing control measures to curb growth and survival of nematodes. Research to evaluate stress resistance provides valuable information regarding the interaction between internal or external stresses and biological processes, such as cellular homeostasis (Park *et al*., 2017).

## MATERIAL AND METHODS

### Sample collection

Since most Plant Parasitic Nematodes (PPNs) are found around the plant roots, collection of rhizosphere samples was carried out in January 2020 from a private farmland in the Village Boriavi, District Anand, Gujarat State (22.61ºN, 72.93ºE). Soil samples were collected from 15–20 cm depth. After collection, the samples were kept moist and were contained in pre-cleaned polythene bags. Samples were stored in a cool, dry place between 16–20ºC. Following sample collection, nematode extraction and purification were carried out using the standard sieving method for free living terrestrial nematodes (Cobb, 1918) and for freshwater nematode identification and purification done by following differential floatation method (Abebe *et al*., 2006).

### Identification and classification

After extraction and purification nematodes were visualized by using Stereo Zoom (Nikon) Research microscope (LM 1080). Images were captured using Motic camera with dedicated Motic Image Plus software. Identification of the nematodes was carried out using two primary dichotomous keys (Tarjan *et al*., 1977; Mekete *et al*., 2012). Nematodes were identified up to genus and species level by succeeding dichotomous key for a particular genus. The following species were identified and used in the stress experiments: *Radopholus* spp., *Aporcelaimus* spp., *Prodontorhabditis* spp., *Eudorylaimus* spp. and *Doryllium minor*.

### Salinity stress assay

Salinity stress assay was performed on cultivated *Radopholus* spp., *Prodontorhabditis* spp. and *Doryllium minor* species following the protocol given

by Hill *et al*. (2014). Nematodes were exposed to different saline solutions having different concentrations of NaCl i.e. 0.25%, 0.50%, 1%, 2%, 4%, 8% and 16% in triplicates, for a duration of 30 minutes at 22ºC and then the washed nematodes were seeded on freshly prepared NGM plates. Nematodes were observed for motility and readings were taken after 1, 24 and 48 hrs. (Hill *et al*., 2014).

### Heat stress assay

Heat stress assay on agar plates seeded with *Radopholus* spp., *Prodontorhabditis* spp. and *Doryllium minor* species was done according to the method stated by Zevian & Yanowitz (2014). The heat stress was induced at 35ºC and 40ºC temperature in a laboratory incubator in triplicates. At each temperature adult worms were exposed for durations of 1 hour, 1½ hour and 2 hours. The exposed nematodes were then maintained at normal cultivation temperature i.e. 30ºC in a separate laboratory incubator for further evaluation of heat stress effect (Zevian & Yanowitz, 2014).

### pH stress assay

The procedure given by Park *et al*. (2017) was followed for the pH stress assay carried out on *Radopholus* spp., *Prodontorhabditis* spp. and *Doryllium minor* species. The nematodes were seeded on nutrient media having different pH range i.e. pH 2, 3, 4, 5, 6, 8, 9 and 10. Nematodes were observed after 1, 24, 48 and 72 hours for evaluation of pH stress effect on test nematodes and results were recorded in triplicate.

As stipulated by Park *et al.* (2017) for each stress assay, active and healthy adult worms were taken for the tests from culture.

### Statistical analysis of the data

Each parameter was expressed as Mean±S.E. The Student's *t*-test was calculated using Microsoft Excel 2010. $EC_{50}$ Values for effective salt concentration for all test species were calculated using HN-NonLin V 1.1 Software (Sharma *et al*., 2016).

## RESULTS

### Identification

The identified five nematode species belonged to five different genera i.e. *Radopholus* spp., *Aporcelaimus* spp., *Prodontorhabditis* spp., *Eudorylaimus* spp. and *Doryllium minor*. Observations carried out indicated that in the selected areas

under study from Gujarat state, infestation of *Prodontorhabditis* spp. was prevalent on green onion (*Allium fistulosum* L.), while in Ginger rhizomes (*Zingiber officinale* Roscoe) *Eudorylaimus* infection was recorded. The presence of *Doryllium minor* and *Radopholus* spp. was observed from root soil of *Trigonella foenum-graecum* L. in Anand district.

## Salinity stress assay

Nematodes selected for evaluation of salinity stress were exposed to different concentration of NaCl and it was observed that *Prodontorhabditis* spp. had transitioned into a state of dormancy due to unfavourable conditions for a period of 1 to 2 hours. However, after 24 hours when the nematodes were transferred to normal growth media, recovery and restoration of normal growth was observed in 0.25% and 0.50% salt concentrations. Hence from the salt stress assay it was evident that the most favourable range of NaCl concentration was found between 0.25–0.50% (Fig. 1).

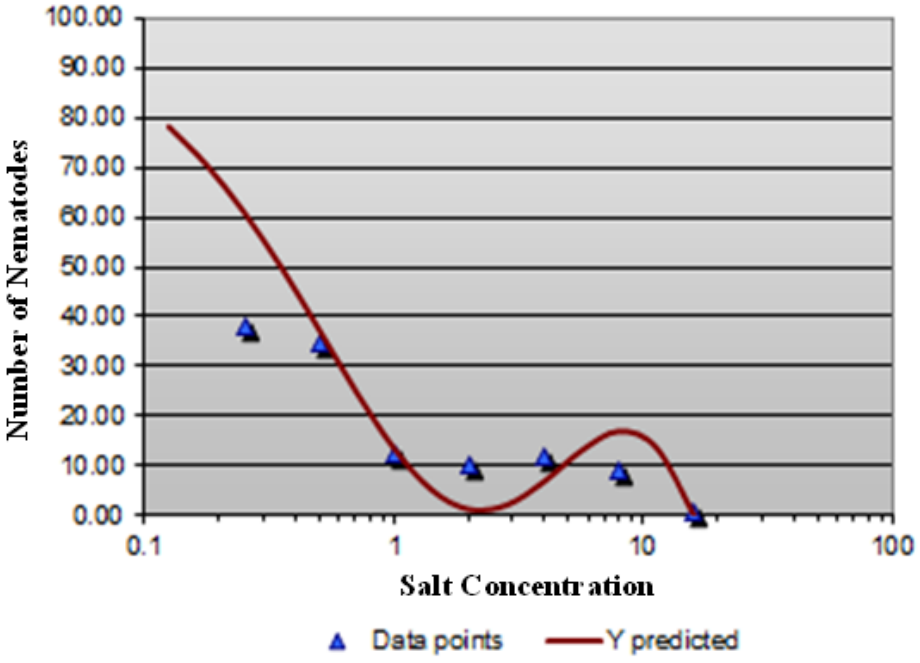


Fig. 1. Salinity Stress Assay on *Prodontorhabditis* spp.

Concentration dependent cessation in motility was also observed for *Prodontorhabditis* spp. after 30 minutes of exposure. A lower recovery rate was noticed. Effective concentration which leads to 50% mortality was observed at

0.35% NaCl concentration. Hence, it was observed that *Prodontorhabditis* spp. was highly sensitive to salinity variations and concentrations of NaCl at and above 0.5% prove unfavourable for this species.

In *Doryllium minor* species although there was a decline in motility observed during the first hour and after 48 hours more than 80% of the nematodes had shown recovery and become motile up to 2% salt concentration. Recovery rate was found to be initiated and increases after 24 hours. *Doryllium minor* showed tolerance to a comparatively broader range of salt concentration i.e. from 0.25% to 0.50% (Fig. 2). $LC_{50}$ for *D. minor* found to be 0.5%.

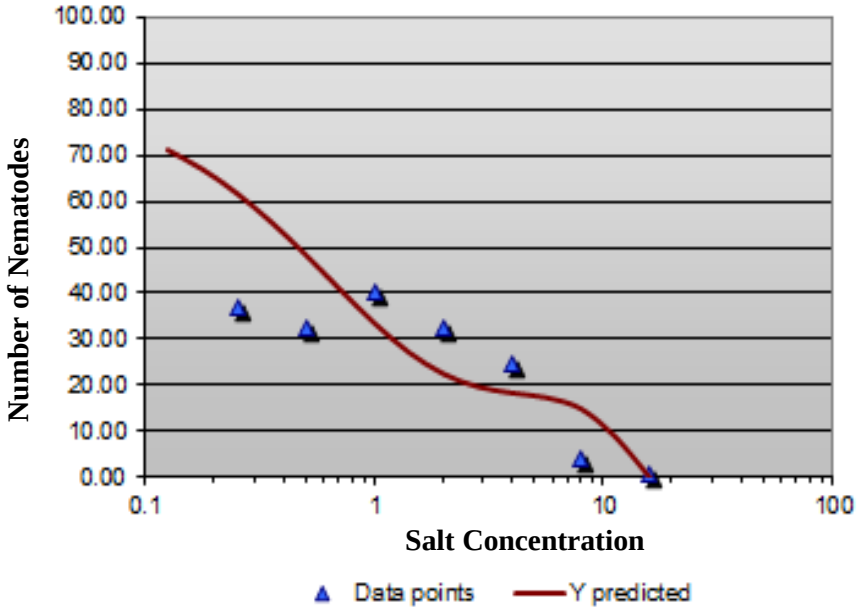


Fig. 2. Salinity Stress Assay on *Doryllium minor*.

*Radopholus* spp. showed best growth and survival at 1% NaCl concentration after 48 hours of recovery. However, the favourable range of salt concentration was observed between 0.25%–2%. Most dormant adults and juveniles were observed up to 24 hours; however after 24 hours recovery rate increases in case of *Radopholus* spp. (Fig. 3). $LC_{50}$ for *Radopholus* spp. found to be 3.73%.

After the Salinity Stress Assay, results obtained from the viability test (Fig. 4) indicated that the favourable range of salt concentration for 3 different treated nematode species. The most favourable salt concentration for *Prodontorhabditis* spp. ranges from 0.25 to 0.50%. Suitable NaCl concentration for *Doryllium minor* ranges from 0.25 to 2% however most growth was observed at 0.25% NaCl

concentration. When cultured in concentrations of 1% NaCl, *Radopholus* spp. retained maximum viability. Hence, all tested nematode species have their unique favourable range of salt environment in which they show maximum growth rate.

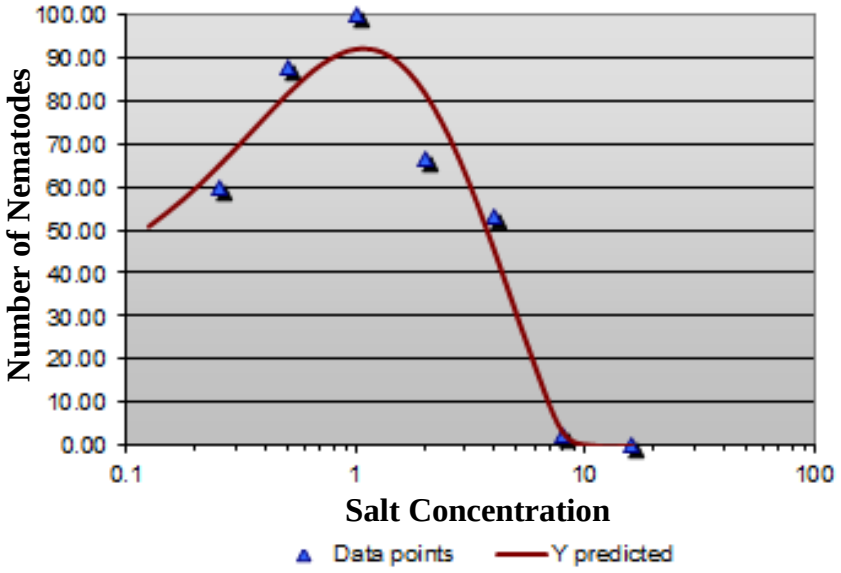


Fig. 3. Salinity Stress Assay on *Radopholus* spp.

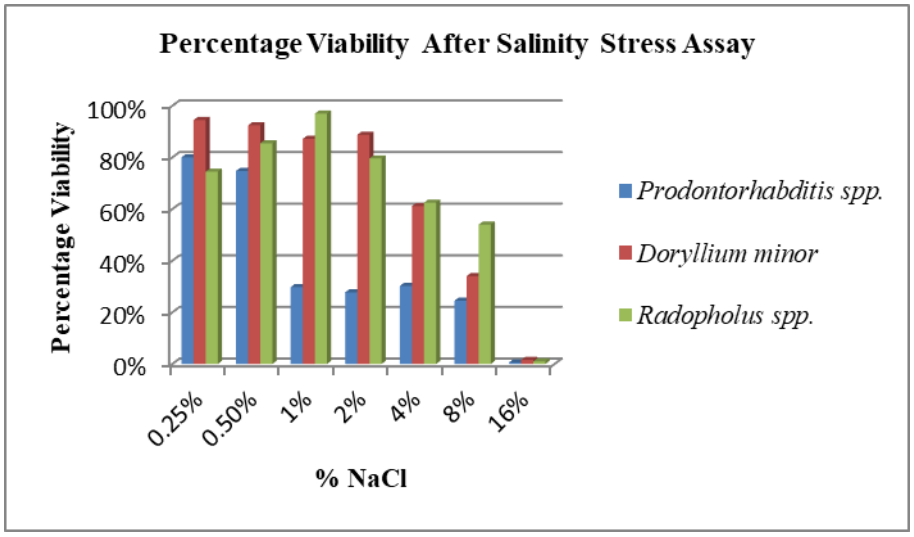


Fig. 4. % Viability after Salinity Stress Assay.

## Heat stress assay

The soil free living nematodes as well as plant parasitic nematodes can tolerate higher temperature fluctuations as compared with freshwater and saline water nematodes. Therefore the temperatures selected for this study were 35ºC and 40ºC, temperatures which are higher than the normal optimum temperature range for soil and plant parasitic nematodes. After subjecting the cultured nematodes to heat shock of 35ºC (Fig. 5) and 40ºC (Fig. 6) for 30 minutes, one hour and two hours, all test species showed dauer formation, retreating to a condition of stasis, as a basic response to stressful conditions. Following heat stress, the feeding rate was found to be increased in case of *Prodontorhabditis* spp. *Doryllium minor*, the ginger parasitic nematode species was able to tolerate higher temperatures. As the time of exposure to heat stress increases the rate of dormancy and rate of dauer formation also increases. After duration of two hours of incubation at 40ºC (Fig. 6), a significant decline in motility was observed. On the other hand, at 40ºC for all the three incubation time durations, almost all nematodes turned dormant. Therefore, temperatures of 40ºC induce heat stress conditions in the nematodes, which appeared to be highly unfavourable temperature for all the test nematodes to withstand.

At 35ºC formation of the dauer larvae was found to be most prominent as an adaptive behaviour found in almost all free living as well as PPNs. However, after stress recovery of dauer larvae to normal motility and viability was observed. The 40ºC heat stress was proven to be most lethal for all tested species within the span of two hours of exposure (Fig. 6). By comparing tested PPNs with free living nematode species, the green onion free living nematode, *Prodontorhabditis* spp. showed better growth, tolerance and survival during and after heat stress for 30 minutes and one hour. Species identified from farmland soil samples, *Radopholus* spp. manifested the least survival and tolerance.

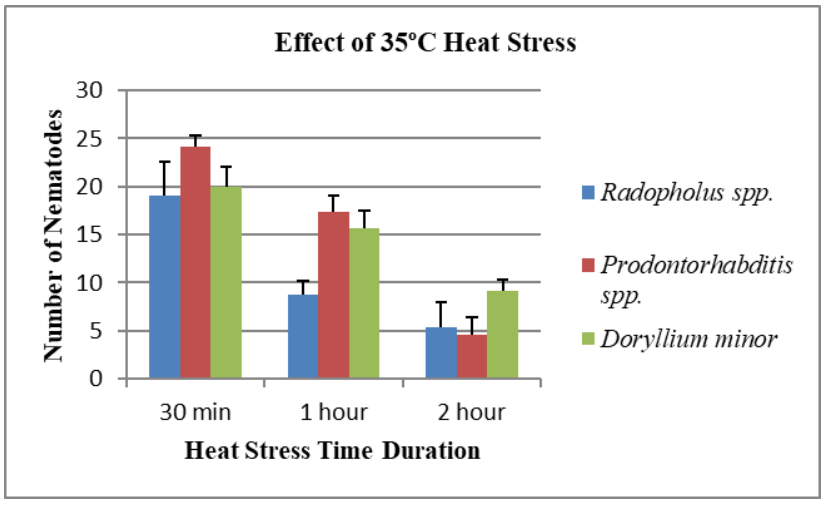


Fig. 5. Heat Stress Assay at 35ºC; Bars represent mean± S.E. (n=6).

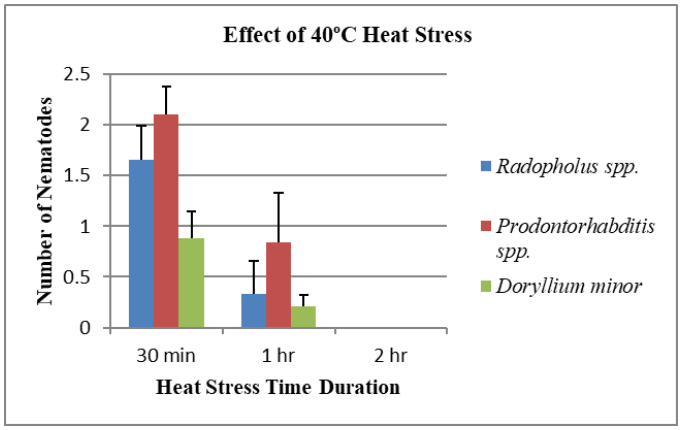


Fig. 6. Heat Stress Assay at 40º C. Bars represent mean± S.E. (n=6).

## pH stress assay

Evaluation of the response of the Nematodes under study to varied pH levels was done employing the pH stress assay on *Radopholus* spp. The stress induces hibernation in all tested nematode species due to change in pH. It was observed that soil nematode *Radopholus* spp. was unable to survive under acidic pH range and showed best growth rate in a basic environment that reflects the adaptation of these species to agricultural soil environment having higher pH range. *Radopholus* spp. showed best growth rate at an alkaline pH between 8 and 9. As shown in Table 1, after 72 hours of incubation in media of varied pH, nematodes showed best recovery in terms of survival and growth at pH 8. As the pH range increases above neutral nematode growth was also found to be increased. While in acidic pH nematodes were unable to survive. At a pH range of 4 to 6, the nematodes showed minimum growth which was less than growth rate found at pH 8.

*Table 1*

pH stress effect and recovery of *Radopholus* spp. at 25º C; Values are mean± S.E. (n=6)

| pH | 1 hour | 24 hour | 48 hour | 72 hour |
|---|---|---|---|---|
| **2** | 29.6±0.92 | 12±3.25 | 4.7±1.07 | 3.7±1.0 |
| **3** | 29.1±0.11 | 8.2±0.08 | 7.5±0.96 | 6.2±1.60 |
| **4** | 25.5±0.90 | 34±5.43 | 38.9±3.67 | 205.1±2.30 |
| **5** | 29.9±1.07 | 75.5±4.90 | 210.1±2.30 | 254.39±1.90 |
| **6** | 26±0.60 | 46.6±1.48 | 397.7±1.89 | 589.1±1.56 |
| **8** | 32.1±2.56 | 50.5±1.23 | 527.4±5.23 | 1052.7±4.17 |
| **9** | 22.5±1.60 | 58.3±1.40 | 422.1±2.13 | 746.9±6.32 |
| **10** | 27.3±0.04 | 41.2±1.45 | 375.2±1.17 | 446±8.60 |

As *Prodontorhabditis* spp. which was identified from samples of green onion, is a slow growing species; the induced pH stress for 48 hours led to a significant decline in the number of nematodes as compared with the *Radopholus* spp. after a similar duration in the pH stress assay. The favourable pH for better growth and multiplication of this species occurred between pH 8 to 9, as shown in the Table 2. Hence soil, free living as well as plant parasitic nematodes have basic pH range favours their growth and multiplication. As shown in the Table 3, the favourable pH range for *Doryllium minor* ranges from 8 to 9. Furthermore acidic pH increases mortality rate in all test nematode species.

*Table 2*

pH stress effect and recovery of *Prodontorhabditis* spp. at 25º C;
Values are mean ± S.E. (n=6)

| pH | 1 hour | 24 hours | 48 hours | 72 hours |
|---|---|---|---|---|
| **2** | 30.23±0.45 | 6.21±0.84 | 2.09±0.37 | 1.08±1.34 |
| **3** | 29.77±0.81 | 8.49±0.54 | 5.18±0.62 | 2.46±0.24 |
| **4** | 31.19±1.26 | 8.73±0.89 | 6.08±0.47 | 2.07±0.92 |
| **5** | 31.04±0.67 | 17.87±1.54 | 24.16±1.04 | 62.15±1.14 |
| **6** | 29.28±0.81 | 23.14±1.29 | 32.89±1.20 | 78.70±0.47 |
| **8** | 30.47±0.91 | 31.84±0.46 | 59.47±0.26 | 98.24±0.57 |
| **9** | 32.54±0.57 | 33.56±0.74 | 60.48±0.82 | 104.08±0.69 |
| **10** | 29.45±1.13 | 30.93±1.25 | 29.15±1.45 | 30.14±1.02 |

*Table 3*

pH stress effect and recovery of *D. minor* at 25º C; Values are mean ± S.E. (n=6)

| pH | 1 hour | 24 hours | 48 hours | 72 hours |
|---|---|---|---|---|
| **2** | 28.23±0.89 | 2.18±1.84 | 1.09±0.41 | 0.71±0.49 |
| **3** | 30.77±0.57 | 4.09±0.58 | 3.18±0.27 | 1.24±1.57 |
| **4** | 29.19±1.26 | 5.63±1.45 | 2.08±0.87 | 1.09±0.49 |
| **5** | 31.45±0.81 | 8.81±0.54 | 4.16±1.17 | 2.15±1.72 |
| **6** | 28.92±0.81 | 24.18±1.67 | 36.79±1.26 | 67.80±0.78 |
| **8** | 30.24±0.57 | 32.48±0.85 | 89.51±0.68 | 127.53±1.67 |
| **9** | 29.73±0.61 | 31.96±0.34 | 57.74±1.81 | 98.08±0.95 |
| **10** | 32.14±1.47 | 27.37±1.46 | 39.41±0.38 | 75.24±1.72 |

## DISCUSSIONS

As suggested earlier, based on research in other organisms by Haverkort *et al.* (1991), our results also indicated that exposure to different stressors could result in multiple biological defects affecting survival, life span, development, reproduction, locomotion and behaviour. The data obtained in the present study also revealed that there was a correlation between the favourable pH range for PPNs with that of the soil and root pH. In addition, results from heat stress assays indicated that the range of temperature required for optimum growth of the nematodes was similar to that found in and around the cultivation field. These observations therefore, provide vital information for establishing ambient conditions requisite for the maintenance of PPN under laboratory conditions, for studying stress pathways in these organisms and for studying the response of the nematodes during altered stress conditions (Duangjan *et al*., 2019). The effect of stress on slow growing (*Prodontorhabditis* spp.) as well as fast growing (*Radopholus* spp.) parasitic nematodes was also observed. This investigation also provides new insights for developing strategies for the natural, chemical-free control of plant parasitic nematodes which attack agricultural fields in large numbers. By altering any one of these parameters and thus inducing stress in the nematodes their survival and growth could effectively be curtailed.

According to the research carried out by Munoz (2003) longevity of *C. elegans* is strongly correlated with heat tolerance and oxidative stress, as after stress assays nematodes were unable to recover damage caused by stresses. In the current investigation, the results obtained for selected nematodes also showed similar correlation between life span and temperature tolerance. However, according to Zhang *et al*. (2021) in some circumstances the enhancement of heat and oxidative stress resistance can be developed which is reflected by prolonged lifespan in *C. elegans* under stress condition with some morphological, physiological changes and genetic mutations. The results obtained following the stress assays reveal that different plant parasitic nematodes manifest different responses towards stress conditions as in case of *D. minor* formation of dauer and the feeding rate was found to be increased after heat stress; a similar behaviour was also performed by *C. elegans* during stress assays.

The stress assays also helped in standardization of species specific cultivation methods. The relationship between longevity and thermal stress was also observed for PPNs and free living *Prodontorhabditis* spp. as well as development of stress resistant progeny after stress was produced same as in case of *C. elegans*. However, this occurred only up to certain temperature limit; at or above 40ºC the nematodes were unable to survive. Induction of heat stress was observed to lead to dauer formation and thermo-tolerant progeny formation which are similar to the study carried out by Munoz (2003). The heat stress assay data also revealed that a temperature of 40ºC proved to be significantly lethal for all soil free living as well

as PPN adults and juveniles. Hence this study provides evidence of thermo-tolerance, a protective behaviour of juveniles and adults, in the species identified from the samples collected from different vicinities in Gujarat.

The mechanisms that activate stress resistance have been elucidated by Munoz (2003). Also thermo tolerance leads to dauer formation after stress assays which lead to morphological changes along with changes in feeding behaviour in nematodes hence producing unhealthy cultures in case of *Prodontorhabditis* spp. Therefore, the range of pH, stress reveals that all tested species have varied favourable range of salt concentration. Favourable pH range for *D. minor* and *Prodontorhabditis* spp. ranges from 8 to 9. *Radopholus* spp. showed best survival and growth at pH 8. Heat and Salt concentration highly need to be focused. From our investigation the highly and precisely favourable range of pH was found to be 8 for *Radopholus* spp. as well as for *Doryllium minor*. However, the precise favourable pH for *Prodontorhabditis* spp. was found to be 9.

## CONCLUSIONS

In summary, all long lived adults are sensitive to different stresses provided to them and also showed favourable range of pH, Temperature and Salt concentration to assist their multiplication and growth. Also, heat stress induced thermo-tolerant progeny was produced in *Prodontorhabditis* spp. In addition, exposure of the animals to thermal stress for a short duration marks an increased resistance to heat and increased longevity. Investigating the effect of salt, heat and pH stress prove significant in supressing the growth of nematodes at certain ranges which can be efficient in PPN control.

*Acknowledgements.* Miss Zankhana R. Pandit gratefully acknowledges the financial support provided by the Government of Gujarat under the Scheme Of Developing High Quality Research (SHODH) (Student Ref. No. 201901380200), Gujarat, India.

## REFERENCES

ABEBE E., ANDRÁSSY I., TRAUNSPURGER W. (Eds.), 2006, *Freshwater nematodes: ecology and taxonomy.* CABI, 31–36.

ATKINSON N.J., URWIN P.E., 2012, *The interaction of plant biotic and abiotic stresses: from genes to the field*. Journal of Experimental Botany, **63** (10): 3523–3543.

BIRD A.F., 1974, *Plant response to root-knot nematode.* Annual Review of Phytopathology, **12** (1): 69–85.

DONG S., QU M., RUI Q., WANG D., 2018, *Combinational effect of titanium dioxide nanoparticles and nanopolystyrene particles at environmentally relevant concentrations on nematode Caenorhabditis elegans*. Ecotoxicology and Environmental Safety, **161**: 444–450.

DUANGJAN C., RANGSINTH P., GU X., WINK M., TENCOMNAO T., 2019, *Lifespan extending and oxidative stress resistance properties of a leaf extracts from Anacardium occidentale L. in*

*Caenorhabditis elegans*. Oxidative Medicine and Cellular Longevity. ID 9012396, 16 pages; https://doi.org/10.1155/2019/9012396

EASTBURN D.M., DEGENNARO M.M., DELUCIA E.H., DERMODY O., MCELRONE A.J., 2010, *Elevated atmospheric carbon dioxide and ozone alter soybean diseases at Soy FACE*. Global Change Biology, **16** (1): 320–330.

HAVERKORT A.J., FASAN T., VAN DE WAART M., 1991, *The influence of cyst nematodes and drought on potato growth. 2. Effects on plant water relations under semi-controlled conditions.* Netherlands Journal of Plant Pathology, **97** (3): 162–170.

HILL A.J., MANSFIELD R., LOPEZ J.M., RAIZEN D.M., VAN BUSKIRK C., 2014, *Cellular stress induces a protective sleep-like state in Caenorhabditis elegans*. Current Biology, **24** (20): 2399–2405.

KUMAR Y., YADAV B.C., 2020, *Plant-parasitic nematodes: Nature's most successful plant parasite*. International Journal of Research and Review, **7** (3): 379–386.

MEKETE T., DABABAT A., SEKORA N., AKYAZI F., ABEBE E. (COMPS)., 2012, *Identification key for agriculturally important plant-parasitic nematodes Prepared for the International Nematode Diagnosis and Identification Course 2012 – A manual for nematology*. Mexico, D.F.: CIMMYT.

MUNOZ M.J., 2003, *Longevity and heat stress regulation in Caenorhabditis elegans.* Mechanisms of Ageing and Development, **124** (1): 43–48.

PARK H.-E.H., JUNG Y., LEE S.J.V., 2017, *Survival assays using Caenorhabditis elegans*. Molecules and Cells, **40** (2): 90–99.

SHARMA S., KAITHOLIA K., MISHRA N., SRIVASTAVA B., PILLAI C.R., VALECHA N., ANVIKAR A.R., 2016, *In vitro sensitivity pattern of chloroquine and artemisinin in Plasmodium falciparum*. Indian Journal of Medical Microbiology, **34** (4): 509–512.

SMIT A.L., VAMERALI T., 1998, *The influence of potato cyst nematodes (Globodera pallida) and drought on rooting dynamics of potato (Solanum tuberosum L.).* European Journal of Agronomy, **9** (2-3): 137–146.

TARJAN A.C., ESSER R.P., CHANG S.L., 1977, *Interactive diagnostic key to plant parasitic, freeliving and predaceous nematodes.* Journal of the Water Pollution Control Federation, **49**: 2318–2337.

ZEVIAN S.C., YANOWITZ J.L., 2014, *Methodological considerations for heat shock of the nematode Caenorhabditis elegans.* Methods, **68** (3): 450–457.

ZHANG Y., LIN L., CUI H., LI B., TIAN J., 2021, *Construction and Application of EGCG-Loaded Lysozyme/Pectin Nanoparticles for Enhancing the Resistance of Nematodes to Heat and Oxidation Stresses.* Foods, **10** (5), 1127.

Received December 26, 2021

**Department of Zoology, Biomedical Technology and Human Genetics, University School of Sciences, Gujarat University-380009, Ahmedabad, Gujarat, India*
e-mail: zankhana3pandit12@gmail.com