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# Biochemical approaches in taxonomic studies on plant parasitic nematodes*

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**Abstract**

The use of biochemical approaches in taxonomic studies of plant parasitic nematodes is reviewed in relation to electrophoresis, isoelectric focussing, 2-dimensional electrophoresis and serology. Emphasis is laid on various biochemical techniques in brief and their application in taxonomic studies on plant parasitic nematodes. The limitations and advantages of these techniques in resolving taxonomic problems and understanding phylogenetic relation among the plant parasitic nematodes are also discussed.

**Key words:** Biochemical techniques, nematode taxonomy.

## 1. Introduction

The utility of biochemical parameters in taxonomic studies was recognized in the beginning of this century by Nuttal[1] when he made biological tests of blood in relation to zoological classification. Much of the work in the first forty years of this century concerned with the immunological determination of similarities and differences. With the introduction of gel electrophoresis and the discovery that genetic information is carried from generation to generation by DNA, the use of biochemical techniques in the study of taxonomy was accelerated. Biochemical techniques are now being applied to resolve taxonomic problems of all types and in many organisms[2-5]. The study of evolution and taxonomy involves an investigation of the changes and variation in the genetic constitution of the organisms. The genetic similarities and dissimilarities can be examined either by structure of genes and through their products. Systematic information can be obtained by examining either base sequence in DNA or the sequence of amino acid protein[6].

Biochemical approach in the taxonomy of nematodes also started in the same fashion as other organisms. In the field of plant nematology, Lee[7] was the first to attempt identification of species of *Meloidogyne* spp. by biochemical techniques. This new approach attempted at that time was considered to be a valuable tool to characterize and study the relationship among plant parasitic nematodes in complimenting and enhancing the information provided by classical and conventional system. There are excellent reviews on this aspect[8-10]

## 2. Importance of biochemical taxonomy in nematology

The conventional system of studies on nematode taxonomy through light microscopy has served well and will continue to do so. Nevertheless, it does have limitations and there is need to resolve taxonomic complexities rather conclusively. We have situations where suitable

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morphological features are sporadic, overlapping, variable, rare or lacking. We have also situations in which similarities in morphology do not necessarily indicate genetic relationship. We have situations where weighing of characters by different individuals is such that confusion and controversies cloud the true genetic relationship. Because of the world-wide importance, *Meloidogyne* is taken as an example to illustrate these observations. To data approximately 53 species have been described and new ones are being described every year. Although most species are adequately described, it is becoming difficult to distinguish between species on the basis of presently available information. Difficulties arise from the considerable variation among individuals of a species in many of the characters now used to distinguish between the species[11]. For example, perineal patterns are quite variable. Measurement of their characters often overlap between species, are environment-dependent and usually many individuals must be examined for a positive conclusion. Furthermore, this method is time-consuming. Mixed populations which occur on a single host and which show pronounced intraspecific variation can present serious difficulties[12]. Host range studies aid in identifying species of root-knot nematode[13], but involve prolonged green-house culturing of differential host plants[14]. The determination of species on the basis of chromosome number has made much progress but requires cytological examination of several specimens and two species may have the same chromosome number. Studies have shown that some *Meloidogyne* reproduce by mitotic parthenogenesis. With that mode of reproduction mutations would be genetically isolated. Thus a multitude of clones could result in further complicating the task of a taxonomist[15]. Scanning electron microscope has revealed certain morphological differences among the *Meloidogyne* spp.[16,17] but most of the characters are in males which are seldom found in natural population and numerous observations are required to identify precisely mixed wild population. Therefore, a few characters remain which can be used by general nematologist for identification of species of root-knot nematodes[18]. Furthermose, the availability of electron microscope in every laboratory is not always possible. Compared to these techniques, the biochemical technique, specially electrophoresis, is useful for obtaining estimates of genetic and stable differences within and between populations. These techniques are particularly suitable for collecting data because they allow the separation and identification of specific soluble enzymes and non-enzymatic proteins. Comparison of isozyme patterns obtained from the electrophoresis of proteins of mass homogenates or from individuals within a population can provide a measure of the similarity between different populations by analysis of shared or different band mobilities[19]. The enzyme banding pattern provides information about the genetic make up of populations. Some enzymes appear to evolve more rapidly than the non-enzymatic protein[20]. Several groups of root-knot nematodes can be identified by this method[21–23]. The species of *Globodera* can be separated biochemically[24–25]. Two pathotypes of *G. rostochiensis* have also been separated by this approach[26] Serological techniques provide reliable procedures for determining differences or similarities in *Meloidogyne* antigen[10]. These techniques have great potential for elucidating phylogenetic relationship and complete understanding of kinds and diversities among the plant parasitic nematodes.

## 3. Methodology

Currently, taxonomic studies on nematode, by and large, involve counting and measuring of

various characters and examining them under different types of microscopes. But the biochemical approach for taxonomic studies involves the techniques borrowed from chemist *e.g.* electrophoresis, isoelectric focussing, 2-dimensional electrophoresis, serology and DNA technology. Many detailed accounts of the theory and practice of these techniques are available in the literature. By analysing the genes it would have been possible to know the variation in the species. But, extraction of nucleic acid and determination of their base sequence are a time-consuming process which require larger amount of material than the generally available from single nematode species[15].

*3.1 Electrophoresis*

Of all biochemical techniques used in taxonomic studies on phytophagous nematodes, the most commonly and widely used method is electrophoresis. Benton and Myers[27] were the first to use electrophoresis to identify soluble protein from the free living nematodes *Panagrellus redivivus* and plant parasitic nematode *Ditylenchus triformis*. In their studies, they found *P. redivivus* has greater number of protein bands than *D. triformis*. Homogenates of *P. redivivus* have at least twenty different protein bands, eight of which exhibit esterases and five acid phosphatase activity. Homogenates of *D. triformis* have at least 16 distinct protein bands of which four exhibit esterase. In view of the high degree of reproducibility of the results and distinct Zymogram and protein patterns obtained in this study they assumed that a taxonomic classification of nematodes based on electrophoresis might be possible.

Subsequent to this study, many attempts have been made to distinguish the different species or races of plant parasitic nematodes with mixed stages of nematode population or large number of individuals[28-33]. Biochemical approaches for nematode taxonomy had to wait till 1978 when Dalmasso and Berge[15] introduced for the first time the use of microtechnique to extract protein from single nematode specimen. They developed microtechniques where acrylamide gels were cast as slabs (0.4 mm thick) or as cylinders in microhematocrit tubes (1.1 mm internal diameter). The micro slab gel technique enables separation of protein from 1 to 10 nematodes on a single gel. Five known and one unknown species of *Meloidogyne* comprising a total of 83 populations from various locations were used in this study. To establish variability in each locus, the electrophoretic analysis of individuals derived from wild populations was replicated 80 times; analysis of individuals from egg mass populations was replicated 20 times. From their studies of protein, esterase, malate dehydrogenase, glycerol phosphate dehydrogenase and catalase, they concluded that non-specific esterases are good tools for taxonomic identification of *Meloidogyne*. This method was further accelerated by Janati[21]. He studied the isoesterase composition of females from 75 different localities and found two types esterases, *b* and $\beta$ after coloration of gels with 1-naphthyl acetate. The distribution of *b*-esterase, is species specific and stable among the population of *M. incognita* and *M. javanica* but some variations were detected among the population of *M. arenaria*. This pattern of *b*-esterase was considered to be a reliable character for identification of *M. incognita, M. javanica* and *M. arenaria*[22]. Polyacrylamide gel electrophoresis has also been employed in order to study intraspecific variations in other nematodes. For example, an attempt has been made recently to differentiate two biotypes of *Heterodera avenae* on the basis of involving non-specific esterase polymorphism[34]. Further-

more, it was of interest to note that genetic polymorphism is pronounced in *H. avenae* as compared to *Meloidogyne* species as far as non-specific esterase is concerned. Recently, starch gel electrophoresis was employed to analyze the genetic variation of banana and citrus race of *Radopholus similis*[35]. It was demonstrated that aldolase, $\alpha+\beta$ esterase, glucose 6-phosphate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase and phosphogluco isomerase were diagnostic marker of the races. Three species of *Bursaphelenchus* were readily distinguishable on the basis of Isoesterases and malate dehydrogenase using polyacrylamide gel electrophoresis[36].

### *3.2 Isoelectric focussing*

Polyacrylamide gel electrophoresis was employed earlier to separate species of the genus *Globodera*. Results are often inconclusive and inconsistent[37,38]. Conventional electrophoresis separates and characterizes protein by relative mobility but the sensitivity, resolution and reproduction of the result can be enhanced by separating proteins to their isoelectric points[39]. The advantage of this method is its reproducibility and the technique overcomes the differences in gel composition, purity of chemicals and running condition of the electrophoresis. This method can detect protein and isozyme differences of *Globodera* spp. and more work may provide a method of diagnosing field population based on this technique[40]

Fleming and Marks[24] found consistent differences for *G. rostochiensis* and *G. pallida*. In their opinion, two bands were useful in species identification. A major protein band at PI 5.9 was found only in *G. rostochiensis* whereas *G. pallida* possessed band with PI values of 5.7. Both the bands could be identified from the extract of single cyst. They are of the opinion that sensitivity of this technique will allow quick and accurate species identification of a single viable nematode cyst from field or consignment of potatoes. Wharton *et al*[25] studied the differences of *G. rostochiensis* and *G. pallida* using the same technique but with acid phosphatase enzyme. The population of the former showed a major band at PI 5.73 which was not detected in three populations of *G. pallida*. On the basis of statistical analysis it was suggested that bands at PI 5.73, 5.88, 5.96 and 6.08 were the most diagnostic in separating *G. rostochiensis* from *G. pallida*. They concluded that this method is good for distinguishing these two species. Fox and Atkinson[40] observed a major protein band at pH 8.0 on the basis of which two species could be differentiated. Lawson *et al*[23] reported that *M. incognita, M. arenaria, M. javanica* and M. hapla can be distinguished from each other by this method with nematodes egg protein. They also got distinct protein profile in the larvae and adults of *Hopolaimus columbus* and H. glycines. Recently this technique provided characteristic protein pattern for ten species of *Heterodera* indicating several races and new species[41].

### *3.3 Two-dimensional electrophoresis*

Separation of proteins consisting cellular and subcellular fractions from a complex mixture sometimes is not accomplished by one-dimensional electrophoresis. The combination of two different electrophoresis to produce a two-dimensional separation increases the resolving power by many folds[42]. O'Farrell[43] introduced two-dimensional electrophoresis where proteins are separated by isoelectric focussing in the first dimension and according to the molecular weight in a sodium dodcyl sulfate pour gradient gel in the second dimension. It is very suitable for detecting low quantity of protein using ultra-sensitive silver stain[44].

Two-dimensional electrophoresis in combination with sensitive protein stain based on a reaction with silver ions allowed differentiation of two closely related species of *Globodera*[26]. Twenty-five micrograms of protein were used in this study. They concluded that two populations belonging to the pathotype $RO_1$ and $RO_2$ of *G. rostochiensis* differred in one protein. But Huettel *et al*[45] reported that the resolution of protein was poor while comparing two races of *Radopholus similis*. Apparently, the protein concentration was too low to detect the components with coomassie brilliant blue. Many protein spots were stained with silver stain but intense background staining obscured them. Large quantities of concentrated protein are necessary to get good results. It is presumable that silver staining procedure has to be adjusted in each case to obtain good result. Premachandran *et al*[46] utilised this technique to differentiate species of *Meloidogyne* and remarked that it may be useful for taxonomic purposes.

### *3.4 Serology*

Serological technique has been employed recently to determine differences or similarities among soluble proteins of some nematode species and has been used extensively to identify bacteria, viruses and fungi. The specificity of the antigen-antibody reaction serological technique is regarded to be reliable in determining homologies between proteins of different animals or plant species[47]. Like electrophoresis, many attempts have been made in determining the differences or similarities among antigens obtained from nematodes. Gibbins and Grandison[48] attempted an assessment of serological procedure for differentiation of biological races of *D. dipsaci*. The number of lines appearing with *D. dipsaci* from lucerne, white clover and narcissus were less than red clover race antigen sample. In their opinion, the use of this technique for differentiating biological races of *D. dipsaci* may be possible if methods are derived for separating the different stages into large pure sample. The results of EL-Sherif and Mai[49] indicated that *P. redivivus* and *Diplogaster* spp. are closely related agreeing with their taxonomic position in the Phylum Nematoda. *Aphelenchus avenae* which is not related taxonomically to the other two nematodes is serologically related to them. They said that in such experiments cross-absorption procedure and healthy serum should include in control. Webster and Hooper[50] identified two distinct serological groups among six species of *Heterodera* and found that three species of *Ditylenchus* serologically distinct. Scott and Riggs[51] showed that two races of *H. glycines* were unrelated to *H. betulae*. The large number of common precipitin band that formed in the double diffusion tests indicated that a close relationship between *M. incognita* and *M. arenaria*. The results comparing antigens of *M. hapla* and *M. javanica* with *M. incognita* and *M. arenaria* suggest that *M. javanica* is more closely related to *M. incognita* and *M. arenaria* than *M. hapla*[8]. Misaghi and McClure[14] observed that egg and larvae of three species of *Meloidogyne* can be separated serologically. The larvae and eggs of *M. incognita* possessed one specific precipitin band not present in *M. javanica* and *M. arenaria*. They suggested that since large quantities of eggs and larvae are readily available for volume production of fluorescently-lebelled antisera, rapid identification of single specimen of root-know nematode could be possible by this approach. Riggs *et al*[52] found that *H. betulae* are distantly related to *H. glycine, H. lespedazae, H. trifolii* and *H. schachtii*. In general, the attempts of serological identification of nematodes are in infancy but are encouraging. In most of the cases, antisera were prepared

from nematode homogenates. Future studies should include the preparation of antisera from purified nematode protein which may improve the efficacy of the approach. While serology is recognized as a powerful tool to understand the relationship between and amongst many organisms, their application in nematology is too limited to comment on. There are instances in the literature which indicate the failure of this technique in differentiating some species of plant parasitic nematodes. For example, two populations of *M. incognita* from Taiwan and Peru could not be distinguished[53]. Scott and Riggs[51] failed to detect the differences in two races of *H. glycines.*

### 3.5. Other methods

Besides these methods, little attention has been given to the other methods for this purpose. Krusberg *et al*[54], while comparing the lipid and fatty acid composition in females and eggs of *M. incognita* and *M. arenaria,* found qualitative differences. Fatty acid composition of *Caenorhabditis elegans* and *C. briggsae* differred qualitatively[55].

Recent advances in DNA technology now permit the rapid and reliable characterization of the genome. The characteristics of nematode DNA like genome size, percentages of guanine-cytosine and repetitive DNA restriction fragment length differences have been employed in the cases of animal parasitic and free-living nematodes[56-60].

Curran *et al*[61] recently used this technique to differentiate *M. javanica* and *M. arenaria.* With the development of ultramicrotechnique for isolation of nematode DNA, purification and characterization of classes of DNA is likely to be a useful tool in separating plant parasitic nematodes at generic and specific level.

## 4. Problems

The foregone review indicates that chemotaxonomy at present is confined to three genera, namely *Meloidogyne, Heterodera* and *Globodera.* The world-wide economic importance, availability of living females at ease and distinctiveness of each life stages are the main reasons for these observations. Furthermore, the micro methods of biochemical techniques can be easily employed in these group of nematodes. Until now, very little work has been done on ectoparasites. A breakthrough in this area of investigations is possible only through *in vitro* culturing of plant parasitic nematodes in large number. In this connection, it may be mentioned that the failure of the researchers to detect enzymes from the homogenates does not necessarily mean absence of these macromolecules because the number of nematodes used earlier could be insufficient for this kind of investigation.

There are contradictory views regarding the interference of host protein on the biochemical compounds selected for taxonomic studies. According to Greet and Firth[33] since the females feed continuously on either unchanged or modified protein the extract of the same would interface with gel banding. The observation with freshly hatched larvae gave reproductive result. Ishibashi[62], also holds the same view but other investigators found no variation[31], influence of host may be limited to certain enzymes[47], the host protein in the nematode gut is so minute that it did not interfere in the study with the races of *R. similis*[43].

It is now well established that certain metabolities are age-dependent. Chow and Paster-

nak[63] demonstrated the occurrence of stage-specific enzyme pattern in *P. silusie*. Dickson *et al*[29] also found differences while comparing several enzyme patterns in different stages of *M. incognita*. This problem can be solved if techniques are available for separating the stages. In the case of *Meloidogyne, Heterodera* and *Globodera* adult and larvae can be isolated easily. Still one has to be careful in explaining data because different points f development in the same stage may have some bearing on chemical characteristics.

Differences in results reported by various researchers may be due to variation in experimental techniques, rather in the investigation itself. Some of the reasons to account for these variations are: (i) collection and storing of nematodes, (ii) methods employed in protein extraction, (iii) storage time of protein extract, (iv) method of enzyme analysis. All these may have profound effect on investigation on biochemical taxonomy and this should be taken into account in future. Many of the problems of variation could be avoided by the use of eggs and larvae rather than females and culturing the nematode on one host.

## 5. Perspective

The important features of a classification are that it should be convenient and reliable in use and provide a defined set of information for each unit classified. A simple key of information would simplify the work of taxonomy which should be based on a set of keys rather than on the phenotypic complexities controlled by an associate with the key. Candidates for the key include the nucleic acids and proteins and probably other macromolecules. Protein is a primary product of structural gene and may be considered to be marker for that gene. As genes are connected into genetic system, a protein may become marker for that system which may be a chromosome or the genome as a whole. Hence by considering a sufficient number of protein markers, the structure of genome can be studied to a considerable degree. For analyzing intraspecific relationship, it is necessary to study those proteins which can exist in multiple form. Electrophoretic techniques are used to detect differences in such polymorphic proteins in different animals. As protein composition is genetically determined, it is not affected by environmental conditions. Macromolecular analysis as a tool in taxonomy is in early experimental phase. As the methods become more reliable, convenient and standard, they will undoubtedly make greater contribution towards solving many problems and facilitate identification.

Physiological and biochemical methods not only aid in corroborating the findings of nematode taxonomist but also make it possible to identify albeit important differences and similarities. They permit phylogenetic explanation at molecular level. With the development of standard cultures to provide quantities of nematode material, a biochemist will be in a position to include information form this unique phylum into fundamental phylogenic concept[64]. Identification of plant parasitic nematode by biochemical methods is in its infancy stage. So caution should be taken to understand fully the biochemical character before using this new approach. It should be applied in no case to well defined taxa[65]. Nevertheless, it should be stressed here that biochemistry does not provide any greater importance than the traditional method of identification. Both sets of data should be incorporated in whole of taxonomic position.

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**References**

1. NUTTAL, G.H.P. *Proc. R. Soc. Lond.* B, 1901, **69**, 150.
2. HALL, R. *Bot. Rev.* 1969, **35**, 285.
3. AVISE, J.C. *System. Zool.* 1974, **23**, 465.
4. WRIGHT, C.A. *Biochemical and immunological taxonomy of Animals,* Academic Press, London, 1974, p. 490.
5. SMITH, P.M. *The chemotaxonomy of plants,* Edward Arnold, London, 1976, p. 313.
6. FERGUSON, A. *Biochemical systematics and evolution,* Blackie, Glasgow, 1980, p. 194.
7. LEE,S.H. *Nematol.* 1965, **11**, 41.
8. HUSSEY, R.S. *Helminth. Abstr.* 1979, **48**, 141.
9. PLATZER, E.G. In *Plant parasitic nematodes,* Vol. III, Ed. B.M. Zuckerman, W.F. Mai and R.A. Rohde, 1981, Academic Press, N.Y., 1981.
10. HUSSEY, R.S. In Nematology in Southern United States. *Southern Co-operative Ser. Bull.* 1981, **276**, 50.
11. HEWLETT, T.W. AND TARJAN, A.C. *Nematropica,* 1983, **13**, 79.
12. WHITEHEAD, A.G. *Trans. Zool. Soc.,* London. 1968, **31**, 263.
13. SASSER, J.N. *Madras Agric. Exp. Stn. Bull.,* 1954, **A-77**, 30.
14. MISAGHI, I AND MCCLURE, M.A. *Phytopath.;* 1974, **64**, 698.
15. DALMASSO, A. AND BERGE, J.B. *J. Nematol.,* 1978, **10**, 323.
16. EISENBACK, J.D., HIRSCHMAN, N.H., SASSER, J.N. AND TRIANTAPHYLLOU, A.G. *A guide to the four most common species of root-knot nematode (Meloidogyne species) with a pictorial key,* Coop. Publ. Deps. Plant Pathol. and Genet., North Carolina State Univ. and U.S. Agency Int. Dev., Raleigh, N.C., 1981, p. 48.
17. HIRSCHMANN, H. In *Concepts in nematode systematics* (Ed. A.R. Stone, H.M. Platt and L.F. Khalil), Academic Press, London, 1983, p. 95.
18. NETSCHER, C. In *Concepts in nematode systematics.* (Ed. A.R. Stone, H.H. Platt and L.F. Khalil), Academic Press, London, 1983, p. 197.
19. NEI, M., MARUYMA, T. AND CHAKRABORTY, R. *Evol.,* 1975, **29**, 1.
20. WILSON, A.C., CARISON, S.S. AND WHITE, T.J. *Ann. Rev. Biochem.,* 1977, **46**, 573.

21. JANATI, A. — Contribution a l'etude des esterases chez les *Meloidogyne* (Nematode, Tylenchida), Ph.D. Thesis Univ. Sci. Technol. Languedoc, 1979, p. 84.

22. JANATI, A., BERGE, J.B., TRIANTAPHYLLOU, A.C. AND DAIMASSO, A. — *Revue. Nematol.,* 1982, **5**, 147.

23. LAWSON, E.C. III CARTER, G.E. JR. AND LEWIS, S.A. — *J. Nematol.,* 1984, **16**, 91.

24. FLEMING, C.C. AND MARKS, R.J. — *Ann. Appl. Biol.* 1983, **103**, 277.

25. WHARTON, R.J., STOREY, R.M.J. AND FOX, P.C. — In *Concepts in nematode systematics.* (Ed. A.R. Stone, H.M. Platt and L.F. Khalil), Academic Press, London, 1983, p. 235.

26. BAKKER, J. AND GOMMERS, F.J. — *Proc. Koninklijke Nederlandse Akad. Wetenschappen,* 1982, **85**, 309.

27. BENTON, A.W. AND MYERS, R.F. — *Nematol.,* 1966, **12**, 495.

28. GYSELS, H. — *Nematol.,* 1968, **14**, 489.

29. DICKSON, D.W., HUISINGH, D. AND SASSER, J.N. — *J. Nematol.,* 1971, **3**, 1.

30. TRUDGILL, D.L. AND CARPENTER, J.M. — *Ann. Appl. Biol.,* 1971, **69**, 35.

31. EVANS, A.A.F. — *Int. J. Biochem.,* 1971, **2**, 72.

32. STONE, A.R. AND WILLIAMS, T.D. — *Ann. Appl. Biol.,* 1974, **76**, 231.

33. GREET, D.N. AND FIRTH, J. — *Nematol.,* 1977, **23**, 411.

34. BERGE, J.B., DALMASSO, A., PERSON REVOAL, F. AND THOMAS, D. — *Revue. Nematol.,* 1981, **4**, 99.

35. HUETTEL, R.N., DICKSON, D.W. AND KAPLAN, D.T. — *J. Nematol.,* 1983, **15**, 338.

36. GUIRAN, G., DE, LEE, M.J., DALMASSO, A. AND BONGIOVANNI, M. — *Revue Nematol.,* 1985, **8**, 88.

37. FRANCO, J. — *J. Nematol.,* 1978, **10**, 287.

38. TRUDGILL, D.J. AND PARROTT, D.M. — *Nematol.,* 1972, **18**, 141.

39. RIGHETT, P.G. AND DRYSDELE, J.W. — In *Laboratory techniques on biochemistry and molecular biology,* Vol. 5, ( Work and E. Work), North Holland, Amsterdam, 1976, p. 337.

40. Fox, P.C. and Atkinson, H.J. *Parasitol.*, 1984, **88**, 131.

41. Rumpenhorst, H.. *Mitteilungen aus der Biologischem Bundesanstalt fur Landund Forstwirtschaft Berlin-Dahlem*, 1985, **226**, 64.

42. Dunn, M. and Berghes, H.M. *Electrophoresis*, **1983**, **4**, 97.

43. O'Farrell, P.H. *J. Biol. Chem.*, 1975, **250**, 4007.

44. Oakley, B.R., Kirsch, D.R. and Morris, N.R. *Anal. Biochem.*, 1980, **105**, 361.

45. Huettel, R.N., Dickson, D.W. and Kaplan, D.T. *J. Nematol.*, 1983, **15**, 345.

46. Premachandran, D., Berge, J.B. and Bride, J.M. *Revue Nematol.*, 1984, **7**, 205.

47. Triantaphyllou, A.C. and Hussey, R.S. *OEPP Bull.*, 1973, **9**, 61.

48. Gibbins, L.N. and Grandison, G.S *Nematol.*, 1968, **14**, 184.

49. El-Sherif, M. and Mai, W.F. *Nematol.*, 1968, **14**, 593.

50. Webster, J.M. and Hooper, D.J. *Parasitol.*, 1968, **58**, 879.

51. Scott, H.A. and Riggs, R.D. *Phytopath.*, 1971, **61**, 751.

52. Riggs, R.D., Rakes, L. and Hamblen, M.L. *J. Nematol.*, 1982, **14**, 188.

53. Hussey, R.S. *J. Nematol.*, 1972, **4**, 101.

54. Krusberg, L.R., Hussey, R.S. and Fletcher, C.L. *Comp. Biochem. Physiol.*, 1973, **45B**, 335.

55. Hutzell, P.A. and Krusberg, L.R. *Comp. Biochem. Biophys.*, 1982, **43B**, 517.

56. Behme, R. and Pasternak, J. *Can. J. Genet. Cytol.*, 1969, **11**, 993.

57. Sulston, J.E. and Brenner, S *Genetics*, 1974, **77**, 95.

58. Kilejian, A. and MacInnis, A.J. *Rice Univ. Stud.*, 1976, **62**, 161.

59. Pasternak, J. and Barrell, R. *Genet. Res. Camb.*, 1976, **27**, 339.

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|---|---|---|
| 50. | Emmons, S.W. Klass, M.R. and Hirsh, D. | *Proc. Natn. Acad. Sci.*, USA, 1979, **76**, 1333. |
| 51. | Curran, J.B. Baillie, D.I., and Webster, J.M. | *Parasitol.*, 1985, **90**(1), 137. |
| 52. | Ishibashi, N. | *Appl. Ent. Zool.*, 1970, **5**, 23. |
| 53. | Chow, H.H. and Pasternak, J. | *J. Expl. Zool.*, 1969, **170**, 77. |
| 54. | Hansen, E.L. and Buecher, E.J. | *J. Nematol.*, 1970, **2**, 1. |
| 55. | Stone, A.R. | *Nematol.*, 1977, **23**, 273. |