**Development of crop management strategies for management of stubby root nematodes (*Trichodorus* and *Paratrichodorus* spp) associated with docking disorder in Sugar beet (*Beta vulgaris*)**

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**Table of Contents**

[**Abstract** iii](#_Toc110341268)

[**Declaration** iv](#_Toc110341269)

[**Acknowledgements** v](#_Toc110341270)

[**1.0 Literature Review** 1](#_Toc110341271)

[**1.1 Introduction** 1](#_Toc110341272)

[**1.2 The family Trichodoridae** 1](#_Toc110341273)

[**1.2.2 Distribution and occurrence of family Trichodoridae in UK** 6](#_Toc110341274)

[**1.2.3 Characteristics of species reported in the UK** 7](#_Toc110341275)

[**.1.2.4 Life cycle** 9](#_Toc110341276)

[**1.2.5 Ecology, Survival and damage** 11](#_Toc110341277)

[**1.2.6. Host range** 15](#_Toc110341278)

[**1.2.7 Damage and symptoms to host crops** 19](#_Toc110341279)

[**1.2.8 Docking disorder in sugar beet** 21](#_Toc110341280)

[. **1.3 Management options for Stubby root nematodes** 23](#_Toc110341281)

[**1.3.1 Introduction** 23](#_Toc110341282)

[**1.3.2 Use of cover crops for the suppression of plant parasitic nematodes (PPN)** 26](#_Toc110341283)

[**1.3.3 The use of brassicaceous crops for the PPN management** 28](#_Toc110341284)

[**1.3.4 Allelopathic plant species** 44](#_Toc110341285)

[**1.3.5 Fungal endophytes in PPN management** 50](#_Toc110341286)

[**1.4 Conclusion** 53](#_Toc110341287)

[**2. Data and methods** 54](#_Toc110341288)

[**2.0 Introduction to data and methods** 54](#_Toc110341289)

[**2.1 Experiment 1: Selection of nematode extraction method** 54](#_Toc110341290)

[**2.2 Experiment 2: Host status experiment on potential cover crop species** 55](#_Toc110341291)

[**2.2.1 Nematode extraction and quantification** 57](#_Toc110341292)

[**2.3 Evaluation of potential of biofumigant cover crops under Field conditions** 58](#_Toc110341293)

[**2.3.2 Field soil sampling** 58](#_Toc110341294)

[**2.2.3 Nematode extraction, Identification and quantification** 59](#_Toc110341295)

[**2.4 Statistical analysis** 59](#_Toc110341296)

[**2.5 Results** 60](#_Toc110341297)

[**2.5.1 Selection of extraction method** 60](#_Toc110341298)

[**2.5.2 Experiment 2: Glasshouse host status experiment** 61](#_Toc110341299)

[**2.5.3 Field experiment to evaluate the potential of biofumigant cover crops in the suppression of SRN** 64](#_Toc110341300)

[**2.6 Discussion** 66](#_Toc110341301)

[**3. References** 69](#_Toc110341302)

[**4. Appendices** 84](#_Toc110341303)

[**Appendix A: RDF Evidence report** 84](#_Toc110341304)

[**Appendix B: RDF Action plan** 114](#_Toc110341305)

[**Appendix C: Gannt chart for October 2021 to October 2022** 119](#_Toc110341306)

**List of Figures**

[**Figure 1*:*** The lifecycle of the stubby root nematode. source: (graham stirling & nicol, 2002) 10](#_Toc110342104)

[**Figure 2**: Host status of field crops to different genera and species of stubby root nematodes. Scheme created from https://www.best4soil.eu/database based on research from Wageningen University and research | Field crops, Lelystad. 16](#_Toc110342105)

[**Figure 3:** Host status of green manure crops to different genera and species of stubby root nematodes. Scheme created from https://www.best4soil.eu/database based on research from Wageningen University and research | Field crops, Lelystad. 17](#_Toc110342106)

[**Figure 4**: Host status of vegetable crops to different genera and species of stubby root nematodes. Scheme created from https://www.best4soil.eu/database based on research from Wageningen University and research | Field crops, Lelystad. 18](#_Toc110342107)

[**Figure 5:** Feeding by stubby root nematode, on a root hair through a feeding tube. photograph (Wyss, 1981) , institute of phytopathology, Germany. 20](#_Toc110342108)

[**Figure 6**:Fanged sugar beet root system (Left) verses healthy root system (Right) 22](#_Toc110342109)

[**Figure 7**: Hydrolysis of glucosinolates into isothiocyanates, thiocyanate and nitriles by enzyme myrosinase. 33](#_Toc110342110)

[**Figure 8:** Glasshouse pot experiment on host status of different cover crop species incubated for 8 weeks after planting 55](#_Toc110342111)

[**Figure 9:** Soil sampling of experimental plots at Bury St. Edmunds site, Suffolk 58](#_Toc110342112)

[**Figure 10:** Polytomous key for morphological identification of nematodes in the family Trichodoridae (Decraemer and Baujard, 1998) 59](#_Toc110342113)

[**Figure 11**: Average densities (±SE) of Stubby toot nematodes (SRN) in100g of soil from different samples, using MgSO4.7H2O and Ludox. Vertical bars show the standard error of the mean (n = 3). \*ns = not significantly different (P> 0.05). 60](#_Toc110342114)

[**Figure 12:** Average densities (±SE) of SRN in100g of soil from different samples, using Centrifugal and two flask extraction methods. Vertical bars show the standard error of the mean (n = 3). \*ns = not significantly different (p > 0.05). 61](#_Toc110342115)

[**Figure 13:** Average Stubby root nematodes (SRN densities) in 100g of soil from five sites/ Vertical bars show the standard error of the mean (n = 3). Means with the same small letter are not significantly different (p > 0.05). 62](#_Toc110342116)

[**Figure 14:** Average initial (Pi) and final (Pf) densities of stubby root nematodes (SRN) per pot (n=7) from a glasshouse experiment with 16 different cover crop species and a fallow control. 63](#_Toc110342117)

[**Figure 15:** Average densities (±SE) of SRN per litre of soil, before planting (Pi) and 4 weeks after cover crop drilling. Vertical bars show the standard error of the mean (n = 5). Means with the same small letter are not significantly different according to Turkey HSD (p > 0.05). 64](#_Toc110342118)

[**Figure 16:** Average reproduction factor (RF= density at 4weeks /Initial density (Pi) of Stubby root nematodes (SRN), Vertical bars show the standard error of the mean (n = 5). Means with the same small letter are not significantly different (p > 0.05) according to Turkey HSD. 65](#_Toc110342119)

**List of tables**

[**Table 1**: The proportion of the sugar-beet crop reported to be affected by Docking disorder for each factory area between 1967-1972; the areas from 1968 onwards are those reported affected in June (the month in which most Docking disorder is usually apparent). Source: Cooke, 1973. 23](#_Toc110343300)

[**Table 2**: Glucosinolates nomenclature, source, structure and acronyms (Wathelet et.al., 2004) 30](#_Toc110343301)

[**Table 3**:Effects of Biofumigation on plant parasitic nematode populations using different Brassicaceae species (Fourie *et al.*, 2016) 41](#_Toc110343302)

[**Table 4:** List of cover crop treatments used in a glasshouse experiment to determine host status 57](#_Toc110343303)

[**Table 5**: Cover crop treatments for field experiment 59](#_Toc110343304)

# **Abstract**

Nematodes belonging to the family Trichodoridae (*Trichodorus* and *Paratrichodorus* spp.) are the most economically important free-living nematodes in the UK. They are polyphagous ectoparasites, commonly known as the stubby root nematodes (SRN), that feed on the root tips of plants causing death of the tap root and hardening and thickening of the lateral roots, eventually leading to a stubby /fangy root system. The roots become inefficient in the absorption of water and mineral salts, leading to stunting and yellowing of the crop. In East Anglia, *Trichodorus* and *Paratrichodorus* spp. have been reported in fields where sugar beet grew poorly; young sugar beet seedlings are most susceptible, and present stunted growth and fanged tap roots, which is known as Docking disorder. Certain cover crops have the potential to suppress plant parasitic nematodes where they act either as a poor/non-host, release allelochemicals that are toxic/ inhibitory or provide a niche for antagonistic microbes. The aim of the field study reported here, was to assess the potential of biofumigant cover crop species i.e. *Raphanus sativus* var. *longipinnatus*, *Brassica juncea* var. Brons, *Raphanus sativus* var. Terranova, for use in a crop rotation system for suppression of SRN. Biofumigant cover crops suppress pests and pathogens through the release of isothiocyanates (ITCs) following the hydrolysis of cell bound glucosinolates by the enzyme myrosinase; this reaction occurs following the maceration of biofumigants stems and foliage which are incorporated into the soil. ITCs have been shown to have nematicidal effects against a wide range of plant parasitic nematodes. A field experiment was conducted in SRN naturally infested field near Bury St. Edmunds, Suffolk. Cover crops were drilled in plots and a fallow control included. Soil sampling for SRN was conducted prior to drilling of the cover crops to establish initial SRN densities (Pi) and also sampled four weeks after planting to monitor changes in the SRN densities. SRN densities were significantly lower (P<0.001) in plots drilled with cover crops compared to the fallow control. No significant differences in SRN densities were observed between the three cover crops. The experiment is still ongoing to evaluate the effect of incorporation of brassica leaves and stems at flowering stage, on the population densities of the SRN

# **Declaration**

I Nyambura Mwangi hereby declare that all the work contained in this Specific Degree Registration Report is my own. Any external assistance and information obtained towards the completion of this report has been duly acknowledged or cited with all sources listed in the References section

# **Acknowledgements**

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# **1.0 Literature Review**

## **1.1 Introduction**

Sugar beet (*Beta vulgaris*) is the second most important sugar crop in the world after sugarcane (Ahmad et al., 2017). Sugar is the primary product derived from sugar beet, while the biproduct, pulp, is used as livestock feed. The crop covers 3.7% of total area under crops and supplies 55% of sugar consumed in the United Kingdom (Tzilivakis et al., 2005). Sugar beet is an economically important crop in East Anglia and the East Midlands areas of England, occupying in the region of 105,000 hectares; 15 M t of sugar were produced from the 2018/19 harvest. Plant parasitic nematodes PPNs) are important pests for many crops globally and result in a c. 14% loss of production annually, which equates to losses of £132 billion. The sugar beet crop is no exception, being subject to infection by a variety of different PPN species, including those categorised as free-living. The most common nematodes affecting sugar beet production are beet cyst nematode *(Heterodera schachtii),* root knot nematodes (*Meloidogyne hapla)* and *Meloidogyne chitwoodi*) and nematodes in the family Trichodoridae (*Paratrichodorus* and *Trichodorus* spp) commonly known as stubby root nematodes (Whitehead and Hooper, 1970; Hafez, 1998)

## **1.2 The family Trichodoridae**

Nematodes in the family Trichodoridae Thorne 1935, belong to suborder Diphtherophorina Micoletzky, 1922 in the order Triplonchida (Blaxter *et al.*, 2004). The family is composed of 109 species, plus one subspecies and six genera: *Trichodorus* Cobb, 1913 (64 valid species), *Paratrichodorus* Siddiqi 1974 (26 valid species ), *Nanidorus* Siddiqi, 1974 (seven valid species), *Monotrichodorus* Andrássy, 1976 (4 valid species), *Allotrichodorus* Rodriguez-Montessoro, Sher & Siddiqi, 1978 ( six valid species) and *Ecuadoru*s Siddiqi, 2002 (two valid species), (Asghari *et al.*, 2018). The family Trichodoridae, previously contained one genus, *Trichodorus* sensu lato which was split into genera, *Trichodorus* and *Paratrichodorus* Siddiqi (1974). The genus *Trichodorus* was established by Cobb in 1913, when he described the species *Trichodorus obstutus*. The genus *Paratrichodorus* was also subdivided into three subgenera *Paratrichodorus, Atlantadorus* and *Nanidorus*. Monodelphic species, were included in two new genera: *Monotrichodorus* for species closely related to *Trichodorus*. and *Allotrichodoru*s Rodriguez-M., Sher & Siddiqi, 1978 for species closely related to *Paratrichodorus* (*Atlantadorus*) (Decraemer, 1980). *Dorylaimus primitivus* de Man 1880, was transferred to the genus *Trichodorus* spp and *Trichodorus obstutus* made a synonym of *Trichodorus primitivus* Micoletzky, (1922) (Winfield and Cooke, 1975).

*Trichodorus* (55 species) and *Paratrichodorus* (33 species) are the largest group and occur worldwide. They are didelphic-amphidelphic genera meaning that they have two genital tubes and their uteri are opposed hence the vulva is located near the mid-body. *Monotrichodorus, Allotrichodorus* and *Ecuadorus* are monodelphic–prodelphic meaning that they have one anteriorly directed genital tube and the vulva is located at 60% of the total body length. (Decraemer and Geraert, 2006). The genera *Trichodorus*, *Paratrichodorus* and *Nanidorus* are the most economically important as they have more number of species, virus vector species and have widely distributed species (Asghari *et al.*, 2018). In the genus Trichodorus, 7% of the species (four species) can transmit viruses i.e. *T. primitivus*, *T. similis*, *T. cylindricus* and *T. viruliferus*. In the genus *Paratrichodorus*, 26% (nine species): *P. pachydermus*, *P. anemones*, *P. divergens*, *P. hispanus*, *P. nanus*

, *P. teres*, *P. tuniensis*, *P. allius*, *P. minor* and *P. porosus* are vectors of tobra viruses. In the genus *Nanidorus* spp., 28.5% (2 species) are known to transmit viruses. *Nanidorus minor* has been reported transmitting tobacco rattle virus (TRV) in the USA and pepper ringspot virus (PRV) in South America (Decraemer and Geraert, 2013). *Monotrichodorus*, *Allotrichodorus* and *Eutrichodorus* have not yet been reported as virus vectors and are distributed mainly in Central America and northern part of South America (Decraemer and Robbins, 2007; Asghari *et al.*, 2018)

Nematodes in the genus *Trichodorus* are widespread in North America and Europe and have also been recorded in main continental land masses and island especially in the West Indies and in Australasia (Decraemer, 1995).Their economic importance /damage to crops was first demonstrated by Christie and Perry who described it as *Trichodorus christie*, and named it stubby nematode due to the symptoms it caused on crop roots. Similar damage symptoms observed in other species now apply to all members of the genus *Trichodorus* (Winfield and Cooke, 1975).

**1.2.1 Morphological characteristics of family Trichodoridae**

**Habitus**

Nematode species in the family Trichodoridae are small (0.5 to 1.5 mm. long), they are cylindrical nematodes tapering at the anterior end. The body is thick, and males and females are short and bluntly round (Allen, 1957). They often have ‘cigar shaped’ bodies, especially males and females of *Paratrichodorus* and *Allotrichodorus* spp*.* The males of genera *Trichodorus* and *monotrichodorus* are clearly ventrally curved (Decraemer, 1995)

**Body cuticle.** The cuticle is smooth and marked by lines or punctuations. It is described as a three layered cuticle with thin outer layer, thick middle layer and thinner inner layer under a light microscope, transmission electron microscopy has however revealed eight layers in the cuticle of *Paratrichodorus allius* (Decraemer, 1995). The cuticle is rather loose as it tends to wrinkle as the nematode moves. The cuticle becomes swollen, especially for the genus *Paratrichodorus* spp., when nematode is fixed, as the fixative tends to make the cuticle separate from the body (Allen, 1957; Decraemer, 1995; Decraemer and Geraert, 2006).The swelling is more pronounced in representatives of the *Paratrichodorus* and *Allotrichodorus* genera. These nematodes move slowly for short distances, due to the meromyarian arrangement of the muscle cells that has been observed under electron microscopy, which restricts the nematode to a simple sluggish movement (Decraemer, 1995). This morphological trait has fundamental implications on the biology and the ecology of these genus (Winfield and Cooke, 1975).

**The Pharynx/Esophagus.** The esophagus is tubular and slender expanding gradually to form a conoid swelling at the base. It is divided into three well defined regions i.e. (i) Anterior muscular part/corpus with a lumen with the feeding structure (Onchiostyle) (ii) slender mid part (isthmus), which is surrounded by a nerve ring (iii) a basal bulb that consists of the pharyngeal glands (Decraemer, 1995). The esophagus is made up of five esophangeal gland nuclei. The overlapping of the ventrosublateral oesophageal glands is a basic diagnostic feature used to differentiate *Paratrichodorus* and *Antlantadorus.* In *Paratrichodorus*, the anterior oesophageal glands overlap the intestine while in *Antlantadorus* the posterior gland overlap the intestine dorsally. *Atlantadorus* (cf. Sid diqi, 1974; key on p. 270).(Decraemer, 1980)

**Onchiostyle.** The onchiostyle is described as hollow, slender and tripartite; it is not fully hollow in its length as there is a muscular sheath that lies in the base, which surrounds the new onchiostyle produced when the old one molts (Allen, 1957). The onchiostyle consists of a stylettiform onchium and an onchiophore. Length of the onchiostyle varies between species ranging from 20 -188 µm (Decraemer, 1995). The onchium has a solid tip with no connection to the lumen and it is not axial. Based on structure, it is suggested that the onchiostyle is used for puncturing plant cells, while actual feeding might involve other mechanisms (Allen, 1957).

**Secretory excretory system (SE).** The secretory excretory pore is present in all known species and is located near the nerve ring, mid ventrally ranging from halfway the pharynx to the pharyngo-intestinal junction and rarely occurring with the anterior part of the intestinal region. Posterior location of the SE is regarded as an important diagnostic feature differentiating subgenus or genus, where very few trichodorids have this (Decraemer, 1995).

**Pores.** All known males and females have one pair of subterminal caudal pores (Allen, 1957; Decraemer, 1995), except for *P. weischeri* with two pairs located terminally or sub terminally. In the genera *Monotrichodorus* and some species of *Trichodorus*, the caudal pores lie very close together and is difficult to differentiate as separate units (Decraemer, 1995). The lateral pores in females are valuable in identification as they vary in position and number in different species. The ventromedian pores maybe located near the vulva in some species while they are paired in others. Males of all known species have a lateral cervical pore located near the level of the excretory pore (Allen, 1957).

**Female reproductive system** All *Trichodorus* and *Paratrichodorus* spp. are didelphic, amphidelphic, meaning that they have two genital tubes and their uteri are opposed and vulva is located near mid-body. *Monotrichodorus* and *Allotrichodorus* spp are monodelphic and prodelphic, meaning they have only one anteriorly directed genital tube and vulva is located 60-90% of the total body length. All species of *Trichodorus, Paratrichodorus, Monotrichodorus*, and *Allotrichodorus* are bisexual and have a spermatheca (Decraemer, 1995). In lateral view, the vulva varies in shape, where it may either be a pore, a transverse slit or a longitudinal slit. The vulva has been used as a distinguishing feature especially in the subgenera *Paratrichodorus* (small longitudinal slit), *Antlantadorus* (pore-like) and *Nanidorus* (small transverse slit). (Siddiqi, 1980)**.** Eleven out of twelve *Trichodorus* spp. have female reproductive systems that are didelphic. The ovaries and reflexed and a spermatheca is present. The cutinized pieces surrounding the vulva are frequently valuable diagnostic aids

**Male reproductive system** Trichodorids are monorchic meaning that they possess a pair of outstretched testes. Spermatids are stored in the *Vesicula seminalis* which is located posteriorly in the testis. In trichodorids, the sperm are amoeboid in shape and the variability in the structure of the sperm has been shown to be an important identification feature in the genus *Paratrichodorus.* Most *Trichodorus* and *Monotrichodorus* spp have oval sperm cells with a sausage shaped nucleus. (Decraemer, 1995). The spicule consists of a head (Manubrum), shaft (Calamus) and a membranous extension (Velum). The size (22-87 µm) and the shape are important features that are also used to differentiate species. For example, *Paratrichodorus* species have straight spicules with no transverse strie or bristles on their surface, while *Trichodorus* species have a lot of diversity in shape, and spicule ornamentations. The capsule of suspensor muscles is also an important diagnostic feature, where in *Paratrichodorus* and *Allotrichodorus* they are poorly developed while in *Trichodorus* and *Monotrichodorus* they are well pronounced. (Decraemer, 1995)

Caudal alae or bursa are found in some males of trichodorids. In trichodorids, the bursa is narrow and is unlikely to aid in copulation. The bursa is present in the genera *Paratrichodorus* and *Allotrichodorus* but absent in *Trichodorus*, except for *T. cylindricus* and *T. paracedarus* and absent also in *Monotrichodorus* species (Decraemer, 1995).

The number and position of the supplementary pre-cloacal papillae (SP) in relation to retracted spicule are regarded as distinctive features in species differentiation in males; Males of *Trichodorus* spp have 1 SP, *Paratrichodorus* and *Monotrichodorus* have 2SP and *Allotrichodorus* has 3SP

The main diagnostic characters for genus identification are (i) female reproductive system (didelphic or monodelphic); (ii) length of vagina and size of vaginal sclerotized pieces in lateral view; (iii) presence of advulvar lateral body pores; (iv) presence or absence of caudal alae in male; and (v) degree of development of copulatory muscles and related habitus and capsule of spicule suspensor muscles.(Decraemer and Geraert, 2006)

### **1.2.2 Distribution and occurrence of family Trichodoridae in UK**

Ectoparasitic nematodes *Trichodorus* and *Paratrichodorus* spp in the family Trichodoridae, are widely distributed in light sandy soils in Europe. In a survey conducted in Yorkshire, UK ,700 samples collected contained at least two species of *Trichodorus* spp or *Paratrichodorus* spp (Cooke, 1989). *Trichodorus* spp, *Pratylenchus* and *Tylenchorynchus* were more abundant in samples collected in Docking, Norfolk where sugar beets were patchy and stunted (Whitehead and Hooper, 1970). Out of ninety-eight fields with light sandy soils from eastern England, , *T. pachydermus* Seinhorst occurred in thirty-five, *T. primitivus* (de Man) in twenty-nine, *T. viruliferus* Hooper in thirteen, *T. similis* Seinhorst in nine, *T. cylindricus* in eight and *T. teres* Hooper and *T. anemones* in two each (Whitehead and Hooper, 1970). Similarly, a survey conducted to determine distribution of *Trichodorus* spp in Great Britain in 1976, in cultivated and undisturbed soils, showed that *Trichodorus primitivus* was found in 283 sites out of 792 positive sites and was more cosmopolitan. *Paratrichodorus pachydermus* was the second most prevalent, occurring in 116 sites. In the survey,2894 samples and 720 records from previous sampling were analysed *Trichodorus sparsus* was recorded for the first time in Great Britain during this survey. *T. cylindricus* Hooper, 1962, *T. viruliferous* Hooper, 1963, *T. similis* and *P. teres* Hooper, 1962 were most common in samples from eastern part of Great Britain*; T. velatus* Hooper, 1972 and *P. anemones* Loof, 1965 occurred in few sites, *P. nanus* Allen, 1957 was only found in Scotland, whereas *T. hooperi* was restricted in the south west of England. *T. vanopapillatus* Hooper, 1972 was recorded in one site.(Alphey and Boag, 1976). The frequent occurrence of *Trichodorus primitivus* and *Paratrichodorus pachydermus* has also been reported in a soil sampling exercise conducted to determine the distribution of tobacco rattle virus and virus vector nematodes in Angus, Banff, Berwickshire and Kincardineshire in Scotland. *Trichodorus* spp was recorded in 133 out of 153 soils sampled. *T. primitivus* and *T. pachydermus* were the most predominant species, occurring in about 100 soils while *T. nanus* was recorded in only 5 soils (Cooper, 1971). Although five Trichodorus species were found in Scottish soils, *T. nanus, T. cylindricus* were rare; numbers of the nematodes were small and populations were restricted to soils in which commercial potato crops were rarely grown. In studies conducted to determine the factors affecting docking disorder in sugar beets in England, *Trichodorus* spp were found to be widely distributed, where they were recovered from 75% of samples collected (Cooke, 1973)

### **1.2.3 Characteristics of species reported in the UK**

**Trichodorus primitivus**

*Trichodorus primivitus* (De Man, 1880) isolated from Retford, UK has been shown to closely resemble Dutch specimens, except that the onchiostyle is longer in the English specimen (Allen, 1957; Hooper, 1962). Reproduction is amphimictic and males are equally abundant as females. The species also has a K survival strategy. *T. primitivus* males can be distinguished from other species in the genus Trichodorus by the position of the supplementary papillae, the first located near the proximal end of the spicules, the second one body width anterior to the first and the third one and three-fourth body widths anterior to the second and the presence of three or four ventro median esophangeal papillae. *T.primitivus* males is one of the two species of *Trichodorus* males with three ventro median cervical papillae and three supplementary tail papillae The females are distinguished by longer tails, ventrally positioned caudal pores, an excretory pore which opens at the level of the nerve ring, the presence of three lateral hypodermal pores with one being slightly posterior to the vulva, one at 3 body widths and the final one at 7 body widths anterior to the vulva , , and the shape of the cutinized vaginal pieces (Allen, 1957). ***Trichodorus cylindricus***

*T.cylindricus* is known to occur together with *T.primitivus*, with both species having morphological similarities (Allen, 1957; Hooper, 1962; Winfield and Cooke, 1975)*.T.cylindricus* has alsobeen found occurring together with *Trichodorus teres* at a site in Norwich*.* Males of *T.cylindricus* are differentiated from *T.primitivus* by the presence of caudal alae, striated spicules and difference in shape of the spicule and the gubernaculum The females are distinguished from *T.primitivus* by shape of the vagina and cutinized pieces surrounding the vulva, and one pair of lateral hypodermal pores (Hooper, 1962)

***Trichodorus teres***

*T. teres* is closest to *Trichodorus minor* Colbran 1956 and *Trichodorus christie* Allen,1957. These three species are easily distinguished by pronounced ventral overlap of the oesophagus over the intestine. *T. teres* differs from these two species by four morphological traits which are: longer body and onchiostyle, shape of vulva, which is a longitudinal slit, difference in shape of vagina and cutinized pieces surrounding the vulva in lateral view and position of the excretory pore, which is more anterior (Hooper, 1962)

***Trichodorus viruliferous***

*Trichodorus viruliferous* reproduction is amphimitic with males being abundant. *T. viruliferous* is closest morphologically to*T. primitivus* (de Man, 1880) and *T. similis* (Seinhorst, 1963); it has three ventral cervical papillae anterior to the excretory pore and three ventral tail end supplements but no bursa. *T. viruliferous* is distinguished from *T. primitivus* and *T. similis* through differences in spicule; the spicules of *T.* *viruliferous* have a narrow bend halfway its length. The gubernaculum is similar to that of *T. similis,* but it has a pronounced keel and is longer in proportion to the spicule. The ventral cervical papillae are equidistant while in the other two species the anterior papillae are 1.5 times further from the middle and posterior papillae. The location of the excretory pore is opposite the middle part of the expanded part of the oesophagus as opposed to the two other species where it is positioned at the isthmus. The males and females of *T.viruliferous* have pronounced ventral overlap of the oesophagus over the intestine which distinguished them from *T.primitivus* and *T.similis .*The females also differ by having thickened vulval pieces and different vagina shape.(Hooper, 1963)

***Trichodorus similis***

*Trichodorus similis* has similar morphological characteristics to *T. primitivus*. It has been found to occur together with *Trichodorus primitivus* and *Paratrichodorus pachydermus* (Whitehead and Hooper, 1970). The males of *T. similis* can be differentiated from *T. primitivus* by only two well developed supplementary papillae, the spicules of *T. similis* are more pronounced and muscular and the gubernaculum is dorsally orientated and not in between spicules like *T. primitivus*. The onchiostyle of *T. similis* is shorter than that of *T. primitivus*, meaning that the ventromedian papillae occur more posteriorly in relation to the onchiostyle. The females of *Trichodorus similis* can be distinguished from *T. primivitus* by the presence of one pair of lateral hypodermal pores and the shape of cutinized ring around the vulva. (Allen, 1957)

***Paratrichodorus pachydermus***

The males of *Paratrichodorus pachydermus* Seinhorst are differentiated by presence of the bursa, the number and position of the supplements, where they possess three ventral supplementary papillae (VSP). The first VSP is just anterior to the distal ends of the spicules, the second is at the anterior beginning of the bursa and the third is about two body widths anterior to the second. Also, the presence of a ventral esophageal papilla also distinguishes the males. In females, the position of the excretory pore in females is also a distinguishing feature where the it opens at about the level of the anterior end of the expanded portion of the esophagus. The series of three lateral pores posterior to the vulva also distinguishes them. All these characteristics distinguish *T. pachydermus* from its closest species. Females are characterized by the position of the excretory pore and the series of three lateral pores posterior to the vulva. These characters separate *T. pachydermus* from males and females of which are the most closely related species *T. porosus*, *T. christiei* and *T. atlanticus* (Allen, 1957).

### **.1.2.4 Life cycle**

The lifecycle of nematodes in the family Trichodoridae involves moulting into four juvenile stages before maturity into a male/female adult. The eggs are laid by the female in the soil. Embryogenesis studies for *Paratrichodorus christie* showed that the egg is laid in single cell stage and the first two cleavages are transverse and longitudinal. First juvenile stages were seen after 96 hours and emerged from the egg 100-120 hours after they were laid (Bird, Goodman and Mai, 1968). The juvenile then hatches from the egg and undergoes four moults to become an adult. The lifecycle takes 3-7 weeks depending on prevailing temperatures and the species involved. Optimum temperature for reproduction and development ranges from 16-24°C depending on the species. The development of *Trichodorus* spp was found to be inhibited at 35°C (Rohde and Jenkins, 1957). The optimum temperature for *Paratrichodorus christie* was found to be 22°C and completed its lifecycle 21-27 days, (Winfield and Cooke, 1975), and at 30°C it was able to complete the life cycle in 16-17 days. On the other hand, optimum temperature for *Paratrichodorus porosus*, was found to be 24°C and *Paratrichodorus allius* took 17-18 days at 27°C (Ayala, Allen and Noffsinger, 1969). Influence of host plant in reproduction was shown in a study where *P. porosus* and *P. christiei* were able to reproduce on maize at all temperatures tested between 12-29°C and (18-35°C) under glass house conditions, although the optimum temperature was 24°C (Ayala, Allen and Noffsinger, 1969). The overall length of the life cycle of *Trichodorus viruliferous* was observed in laboratory experiments using apple seedlings grown in cylindrical tubes where 30 gravid females and 20 males were inoculated. Eggs and juveniles were first observed 5 and 19 days respectively after inoculation. After 35 days, the medium size juveniles were more predominant. The first adults appeared after 45 days (Pitcher and Mcnamara, 1970). The proportion of actively breeding females with developed oocytes of *T viruliferous* increased rapidly on inoculation experiments with apple seedlings, this was however not the case of *T viruliferous* from field soil samples collected from a mature apple orchard, which was mostly composed of non-breeding females with undeveloped oocytes. Comparisons made between the roots of apple seedlings used in laboratory experiments and roots of established apples in orchards, showed that the fine fibrous root system found in young apple roots provided a more efficient substrate than the fine feeder roots found in mature apple trees (Pitcher and Mcnamara, 1970). Similar experiment showed that populations increase of *Paratrichodorus christie* followed a sigmoid growth pattern when grown in pots with *Lactuca sativa* seedlings where populations increased from ![Diagram

Description automatically 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ABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQB//Z)6,107/1 after 45 days to 12,533/1 after 90 days (Bird, Goodman and Mai, 1968)

Figure 1***:*** The lifecycle of the stubby root nematode. source: (graham stirling & nicol, 2002)

### **1.2.5 Ecology, Survival and damage**

#### **1.2.5.1 Ecology**

Nematodes in the family Trichodoridae, mostly inhabit sandy soils. The clay particles and silt are said to inhibit movement of trichodorids, hence they occur and thrive in sandy soils (Winfield and Cooke, 1975). The ability of the nematode to move is dependent on the size of the soil pore as well as the free path i.e. the distance a nematode can move without distraction; this is mostly important for long nematodes like longidorids. For shorter nematodes like trichodorids, the body diameter is key as the nematodes are plump, which explains the relationship between the adult body diameter and the occurrence and thriving in course sandy soils where the pore size allows the nematode to effectively move (Winfield and Cooke, 1975). The juveniles of *Heterodera* and *Trichodorus* and the early juvenile stages of *Longidorus* average 20 µm in diameter and are therefore unable to penetrate densely packed soils consisting only of clay, silt or the finer fractions of fine sand with particle diameters less than 50 µm (Jones, Larbey and Parrott, 1969). Evidence of the nematodes’ ability to move in sandy soils with greater pre-spaces was shown in experiments conducted using four different grades of sand. Movement was best in the 200-400µ sand fraction and slightly less in the 100-200 µ and 400-800 µ fractions; the least movement was in the 800-1400 µ fraction (Winfield and Cooke, 1975). Mechanical analysis of ten soils obtained from East Anglia in which *Trichodorus* nematodes were found to be abundant, had a profile of 32-60% coarse sand, 22-42% fine sand, 6-12% silt and 7-12% clay. The organic matter content was low, and the free calcium carbonate was between 1.8 and 4.6%. Soil with fine texture and a high proportion of silt and clay had no *Trichodorus* spp. (Jones, Larbey and Parrott, 1969). In a survey conducted to determine distribution of trichodorids in the British Isles, trichodorids were mainly found (50% of infested sites) in soils with a sand fraction greater than 80% and a less than 10% silt. The remainder of the sites with trichodorids had sandy loamy soils. No trichodorids were obtained in clay or silt soils (Alphey and Boag, 1976). In 98 fields with light sandy soils from eastern England *P. pachydermus* Seinhorst occurred in thirty-five, *T. primitivus* (de Man) in twenty-nine, *T. viruliferus* Hooper in thirteen, *T. similis* Seinhorst in nine, *T. cylindricus* in eight and *T. teres* Hooper and *T. anemones* in two each, showing the wide distribution of *Trichodorus* spp in sandy soils (Whitehead and Hooper, 1970),. *Trichodorus velatus* was isolated from sandy soils with Sitka spruce seedlings and herbaceous plants while *Trichodorus variopapillatus* was isolated from woodland moist sandy soil, planted with elder (*Sambucus nigra* L.). *T. hooperi* was isolated from sandy loam soils in mixed conifer woodland with herbaceous undergrowth (Loof, 1973). In Scotland, trichodorids were detected in 75% of potato fields In fields with high clay, spraing (Tobacco rattle virus) which is transmitted by trichodorids, was not detected. Spraing was constantly detected in fields where some potato varieties had been grown in sandy soils. The frequency of occurrence of *P. pachydermus* and *T. primitivus* varied on different soil series. *P. pachydermus* was mainly found in links and raised beach soils while in fluvioglacial soils it occurred only in the Boyndie series and absent in till soils. *T. primitivus* on the other hand, occurred in raised beaches, till alluvial and fluvioglacial soils and rarely exceeded 50 nematodes in 200 grams of soil. (Cooper, 1971).In sites where docking disorder of sugar beet occurred, in East England, the soil had lower silt and clay fractions; *Trichodorus* spp. were recovered from 75% of soil samples (337 nematodes /Litre of soil. in infested samples) (Cooke, 1973). In a survey of potato fields located in East and west Flanders in Belgium, *T. primitivus*, *T. similis* and *P. pachydermus* were isolated from sandy loam soils. *T. primitivus* was the most predominant species and widely distributed in both East and West Flanders compared to the other *Trichodorus* species (Decraemer, Coolen and Hendrickx, 1979)

In a survey to determine distribution of nematodes around roots of *Ammophila arenaria* and *Desmoschoenus spiralis* on sand dunes, *Trichodorus* spp were not found which led to a conclusion that very course sands may limit *Trichodorus* spp distribution (Yeates, 1967). An exception of occurrence of *Trichodorus* spp in sandy soils is in a survey where *Trichodorus pachydermus* (Cooper, 1971) and *Trichodorus primitivus* were found in a clay soil and wide range of soil types, indicating that the two species can occupy diverse soil habitats (Seinhorst, 1963)

#### **1.2.5.2 Vertical and horizontal distribution**

The vertical and horizontal distribution of *Trichodorus* spp can vary enormously. Unlike other nematode genera, the distribution is influenced by the moisture levels in the soil. When soil is at field capacity, numbers can be maintained especially when there is an abundance of host plant roots. Trichodorids tend to move deeper in the soil during dry conditions, where population densities can decrease rapidly due to their high susceptibility to desiccation (Winfield and Cooke, 1975). In fifteen experiments to study the effect of soil fumigation and nitrogen fertilizers in the management of trichodorids and other plant parasitic nematodes, low numbers of trichodorids were recovered from 0-5cm depth, however when the soil was wet in rainy months, a greater number of trichodorids were found in the top soil layer indicating that increased nematode numbers was correlated with accumulated rainfall; for instance numbers decreased in August when rainfall was lower (Cooke and Draycott, 1971). The ability of trichodorids to move when moisture levels are optimum was shown in a study conducted at four different moisture regimes, where greatest movement occurred when soil pores were half full of water and least when soil was dry or waterlogged (Bor and Kuiper, 1966; Winfield and Cooke, 1975). Under field conditions, sandy soils are free draining and hence waterlogging may not be long lived. In similar studies, effect of soil drying out on migratory nematodes was determined; *Trichodorus* spp were found to be more susceptible to desiccation than *Rotylenchus* spp and *Pratylenchus* spp (Rössner, 1971). Similar studies showed that that the family Dorylaimida was more susceptible to high desiccation and osmotic stress when compared to family Tylenchida (Wyss, 1970). This is also explained by the high drainage of water in sandy soils where the topsoil dries out as water percolates deeper through the soil profile.

Depletion of nitrogen and manganese has also been shown to influence these nematodes. Most sandy soils are depleted in nitrogen and manganese where docking disorder symptoms have been recorded (Whitehead and Hooper, 1970). Deficiency in copper has also been associated with distribution and occurrence of *Trichodorus* spp in different soils where *Paratrichodorus pachydermus* was shown to be sensitive to high copper and manganese exposure and therefore occurred in copper deficient soils. *Trichodorus primitivus* was shown to be able to be tolerant to high levels of copper and possible explanation why it is widely distributed in diverse environments (Cooper, 1971).

In other studies, the number *Trichodorus teres* there were fewer trichodorids at 15-30 cm depth and numbers increased deeper below 30cm in sugar beet fields where the roots vertical growth had seized due to damage by the nematodes (Kuiper and Loof, 1962). The depth at which trichodorids occur also varies depending on nematode species. Findings by Richter (1969), in Germany, showed that the males of *P.pachydermus* and *T.viruliferous* differed in depth of occurrence, where *P pachydermus* males were found deeper. However, studies in England, found no clear difference in the depth where *T.cylindricus* or *P. anemones* was found in the topsoil of infested sugar beet fields, except for one field wherefewer *cylindricus* were found in 0-5 cm depth (Whitehead and Hooper, 1970)

#### **1.2.5.3 Survival and sensitivity to mechanical injury**

Soil moisture is an important factor that affects the survival of trichodorids. Increased nematode densities have been observed in wet months in England. A positive correlation was recorded in severe damage observed in young sugar beet seedlings and the high total rainfall in the month of May (Cooke, 1973).Trichodorids are also very sensitive to mechanical injury associated with sampling and handling. This has been shown in a study where soil carefully transported from field to laboratory yielded more *Paratrichodorus teres* (2240 nematodes l-1 soil) as compared to soil sent via post in a cardboard box which yielded 628 nematodes l-1 soil. The reduction was attributed to manual handling during transportation resulting in the death of a high proportion of nematodes. In this study, the diameter of the sampling auger was also shown to influence the numbers of trichodorids recovered during a sampling exercise. Sampling with a 10cm corer diameter yielded 2540 l-1 soil *P.teres* while 2cm and 1cm corers yielded 580 and 390 l-1 soil respectively; this was explained by the fact that a narrow corer exerts more mechanical pressure during sampling compared to a wider diameter corer (Bor and Kuiper, 1966). The effect of soil sample handling on the survival of the nematodes was also investigated in this study, where the effect of dropping soil from certain heights and mixing the soil was investigated. Significantly higher numbers of nematodes died when soil samples were dropped from 100-350 cm as compared to the control where soil was not dropped. In contrast, mixing of the soil did not cause significant mortality when compared the control (no dropping/mixing) (Bor and Kuiper, 1966). Sensitivity of the nematodes to chemical compounds has also been shown in experiments using CuSO4 or MnSO4. Variability in nematode sensitivity upon exposure to three different concentration, depended on the species. In this experiment, *P. pachydermus, T. cylindricus,* *T primitivus* (Trichodorids) and other soil nematodes were compared. Results showed that Trichodorids were more sensitive than other parasitic nematodes as none of them were mobile after 36 hours exposure time. *P. pachydermus* was the most sensitive to copper and manganese while *Trichodorus primitivus* was the least sensitive. The most copper sensitive species (*Paratrichodorus pachydermus*) was found to mainly occur in calcium and manganese deficient soils. However, further field tests to evaluate the sensitivity of the*Paratrichodorus* spp by artificial application of cupric and manganous sulphate, did not reduce the densities of *Paratrichodorus spp* after 16 months of application (Cooper, 1971).

### **1.2.6. Host range**

The family Trichodoridaehas a wide host range attacking crops in diverse plant families. Host preference varies for different *Trichodorus* and *Paratrichodorus* spp. Overall, trichodorids appear to have a wide host range, with densities being considerably influenced by cropping, this is because nematode numbers in the soil prior to planting a crop may result from the previous crop or sequence of crops in a rotation which might have been good hosts hence proliferating the densities. Knowledge of species present and the host status of the crops in the rotation can therefore assist in maximizing the yields of susceptible crops. In a series of rotations testing the effects of Bermuda grass or bahia (*Paspalum notatum*), *Paratrichodorus christiei* was slightly favoured by a continuous row-crop (cotton-maize-peanut) rotation where cotton (Gossypium herbaceum Linnaeus) and maize (*Zea mays*) increased numbers while peanut (*Arachis hypogea*) supressed the densities. Low densities were recorded in a rotation sequence where cotton and maize did not follow each other after a grass ley. Susceptibility of hosts often differs depending on the species attacking and variations even in species from different populations have been observed. In a host range experiment, peas and spinach were rated as good hosts for *Trichodorus christie* (Riveside isolate), while they were poor hosts for *Trichodorus christie* (Florida isolate). Cabbage was also an excellent host for the Florida isolate while it was ranked a good host for the Riverside isolate showing the difference in host responses due to differences in population densities (Pf or final population). Figure 2-4 below, show the host status of different field crops, vegetables and green manure cover crops to SRN species

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  | Host crop | *Paratrichodorus pachydermus* | *Paratrichodorus teres* | *Trichodorus primitivus* | *Trichodorus similis* |
|  | Barley | Excellent host | Moderate host | Moderate host | Unknown |
|  | Beet (Sugar, Fodder | Excellent host | Excellent host | Moderate host | Excellent host |
|  | Black fallow | Active population decline | Active population decline | Active population decline | Active population decline |
|  | Clover | Unknown | Excellent host | Unknown | Unknown |
|  | Faba bean | Unknown | Unknown | Unknown | Unknown |
|  | Hemp | Unknown | Unknown | Unknown | Unknown |
|  | Linseed | Unknown | Poor host | Unknown | Unknown |
|  | Lucerne | Unknown | Poor host | Unknown | Unknown |
|  | Lupins | Unknown | Excellent host | Unknown | Unknown |
|  | Maize (Corn) | Unknown | Excellent host | Unknown | Moderate host |
|  | Oat | Unknown | Moderate host | Unknown | Unknown |
|  | Potato | Excellent host | Poor host | Moderate host | Excellent host |
|  | Rapeseed | unknown | Excellent host | Excellent host | Unknown |
|  | Rice | Unknown | Unknown | Unknown | Unknown |
|  | Rye | Excellent host | Excellent host | Unknown | Unknown |
|  | Soybean | Unknown | Unknown | Unknown | Unknown |
|  | Sunflower | Unknown | Unknown | Unknown | Unknown |
|  | Tobacco | Unknown | Unknown | Unknown | Unknown |
|  | Triticale | Unknown | Unknown | Unknown | Unknown |
|  | Wheat | Excellent host | Moderate host | Excellent host | Excellent host |

Figure 2: Host status of field crops to different genera and species of stubby root nematodes. Scheme created from <https://www.best4soil.eu/database> based on research from Wageningen University and research | Field crops, Lelystad.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Host crop | *Paratrichodorus pachydermus* | *Paratrichodorus teres* | *Trichodorus primitivus* | *Trichodorus similis* |
| Berseem clover | Unknown | Unknown | Unknown | Unknown |
| Buck wheat | Unknown | Unknown | Unknown | Unknown |
| Camelina/False flax or linseed dodder | Unknown | Unknown | Unknown | Unknown |
| Chick pea | Unknown | Unknown | Unknown | Unknown |
| Crimson clover | Unknown | Unknown | Unknown | Unknown |
| Fodder rape/Leafy | Unknown | Unknown | Unknown | Unknown |
| Italian ryegrass | Excellent host | Excellent host | Excellent host | Excellent host |
| Japanese/black oats | Unknown | Unknown | Unknown | Unknown |
| Marigold | Unknown | Unknown | Unknown | Unknown |
| Mustards | Unknown | Unknown | Unknown | Unknown |
| Perenial ryegrass | Excellent host | Excellent host | Excellent host | Excellent host |
| Persian reversed clover | Unknown | Poor host | Unknown | Unknown |
| Phacelia | Moderate host | Unknown | Poor host | Unknown |
| Radish | Moderate host | Poor host | Excellent host | Moderate host |
| Ramtil | Unknown | Unknown | Unknown | Unknown |
| Redclover | Unknown | Unknown | Unknown | Unknown |
| Sorghum Sudan grass | Unknown | Unknown | Unknown | Unknown |
| Turnip | Unknown | Unknown | Unknown | Unknown |
| Vetch | Unknown | Unknown | Excellent host | Unknown |
| White clover | Unknown | Excellent host | Unknown | Unknown |
| White mustard | Excellent host | Unknown | Excellent host | Excellent host |

|  |  |  |
| --- | --- | --- |
|  |  |  |
|  | Legend damage | |
|  |  | Unknown |
|  |  | None |
|  |  | Little (0-15%) |
|  |  | Medium |
|  |  | Serious |
|  |  |  |

Figure 3: Host status of green manure crops to different genera and species of stubby root nematodes. Scheme created from <https://www.best4soil.eu/database> based on research from Wageningen University and research | Field crops, Lelystad.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Host crop | *Partrichodorus pachydermus* | *Paratrichodorus teres* | *Trichodorus primitivus* | *Trichodorus similis* |
| Asparagus | Unknown | Unknown | Unknown | Unknown |
| Basil | Unknown | Unknown | Unknown | Unknown |
| Beans | Excellent host | Moderate host | Excellent host | Unknown |
| Black salsify | Poor host | Moderate host | Unknown | Moderate host |
| Cabbage (Incl. Cauliflower and Broccoli | Unknown | Excellent host | Excellent host | Unknown |
| Carrot | Moderate host | Moderate host | Moderate host | Poor host |
| Celery | Unknown | Moderate host | Unknown | Unknown |
| Chicory | Moderate host | Moderate host | Unknown | Moderate host |
| Coriander | Unknown | Unknown | Unknown | Unknown |
| Corn salad | Unknown | Unknown | Unknown | Unknown |
| Cucumber | Unknown | Unknown | Unknown | Unknown |
| Dill | Unknown | Unknown | Unknown | Unknown |
| Eggplant | Unknown | Unknown | Unknown | Unknown |
| Garlic | Unknown | Unknown | Unknown | Unknown |
| Leek | Unknown | Poor host | Unknown | Poor host |
| Lettuce | Unknown | Unknown | Unknown | Unknown |
| Melon | Unknown | Unknown | Unknown | Unknown |
| Onion | Non-host | Moderate host | Excellent host | Unknown |
| Parsley | Excellent host | Unknown | Unknown | Unknown |
| Parsnip | Unknown | Unknown | Unknown | Unknown |
| Peas | Unknown | Poor host | Poor host | Poor host |
| Pumpkin | Unknown | Unknown | Unknown | Unknown |
| Rhubarb | Unknown | Unknown | Unknown | Unknown |
| Rocket | Unknown | Unknown | Unknown | Unknown |
| Spinach | Excellent host | Poor host | Poor host | Unknown |
| Strawberry | Unknown | Unknown | Unknown | Unknown |
| Sweet pepper | Unknown | Unknown | Unknown | Unknown |
| Tomato | Unknown | Unknown | Unknown | Unknown |
| Watermelon | Unknown | Unknown | Unknown | Unknown |

Figure 4: Host status of vegetable crops to different genera and species of stubby root nematodes. Scheme created from <https://www.best4soil.eu/database> based on research from Wageningen University and research | Field crops, Lelystad.

### **1.2.7 Damage and symptoms to host crops**

#### **1.2.7.1 Feeding:**

Trichodorids are commonly known as stubby root nematodes due to the characteristic symptom that they cause when they feed on the roots. The nematodes feed externally as they are ectoparasitic and adhere closely to the roots causing injury to the root tips and hence stunting root growth (Christie and Perry, 1951; Whitehead and Hooper, 1970). Studies have shown the aggregation phenomenon in *Trichodorus viruliferous* on apple tress via direct observations, cinematography and soil and root sampling. The striking feature is the aggregation of the nematodes around the elongating zone of young roots (Pitcher and Mcnamara, 1970).*Trichodorus* spp were also seen to aggregate around the roots of sugar beet seedlings during surveys in East England (Whitehead and Hooper, 1970). As a result of damage to the root system plants are unable to absorb enough nutrients, and leaves may show symptoms of nitrogen or magnesium deficiency (Whitehead and Hooper, 1970; Winfield and Cooke, 1975; Cooke, 1989). Feeding mainly occurs on the epidermal tissues of young seedlings, often the tap root stops growing or is killed and lateral roots near the surface thicken and replace it resulting in a poorly yielding misshapen (fangy) root at harvest. (Whitehead and Hooper, 1970). At Gayton, Thorpe, England, *Trichodorus* spp especially *Trichodorus cylindricus* or *Trichodorus pachydermus* were common mostly around young seedlings (1500/Litre soil) than around large plants ( 600/Litre soil) (Whitehead and Hooper, 1970).The stubby lateral roots later turn grey- brown and then black as they die and decay.(Christie and Perry, 1951). The feeding is divided into four phases: Exploration, penetration, salivation, ingestion, and withdrawal. During exploration the lips of the nematode rub the plant cell wall to find a suitable cell, upon location of suitable cell, the lips are pressed against the cell wall and penetration involves several thrusts of the onchiostyle. Subsequent to penetration, rate of thrusting drops and the cell cytoplasm streams and accumulates into the feeding site, the nematode therefore ingests large volumes of the cytoplasm through retraction of the onchiostyle

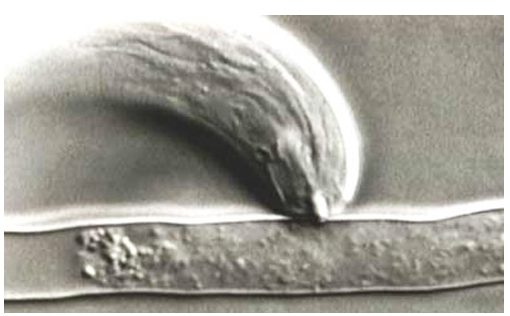
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Figure 5: Feeding by stubby root nematode, on a root hair through a feeding tube. photograph (Wyss, 1981) , institute of phytopathology, Germany.

After ingestion of all cytoplasm, the nematode withdraws the onchiostyle, leaving behind a feeding tube attached to the cell wall (Wyss, 1981). Rasping during feeding has been reported for *Paratrichodorus minor* (Rohde and Jenkins,1957, Russell and Perry 1966).T.similis was observed to feed on upto 15 cells in an hour where it fed on an individual cell for few minutes and moved to the next cell Males and females fed the longest where 2hrs and 50 mins feeding time was recorded (Wyss, 1981). Although a shallow root system is a common symptom associated with injuries by *Trichodorus and Paratrichodorus* spp*,* effects differ because of the influence of secondary pathogens or soil conditions. A patchy appearance due to damaged plants is visible in an infested field; less damaged plants may recover resulting in a 'hen and chick' effect in the field (Sykes and Brown, 1971). The degree of attractiveness of roots to Trichodorids determined the numbers found in the soils taken near the root system (Whitehead and Hooper, 1970). Feeding involves continuous thrusting and withdrawal of the stylet causing large brown lesions to form, while repeated feeding at the root tip inhibits further growth and leads to the formation of browning (Decraemer and Robbins, 2007). *T. proximus* caused chlorosis to St. Augustine grass and reduced the growth; root weight was greatly reduced by the nematode. An examination of infected roots by *T.proximus* showed that lesions were irregular in shape and were deeper in the root tissues (Rhoades, 1965). Studies conducted to determine the pathogenicity of *Paratrichodorus christie* on onions showed that symptoms caused by the nematodes on onion, were as a result of longitudinal and radial increase of the cortex and that earlier damage may have resulted from abnormal cell maturation near the apical meristems (Hoff and Mai, 1962)

### **1.2.8 Docking disorder in sugar beet**

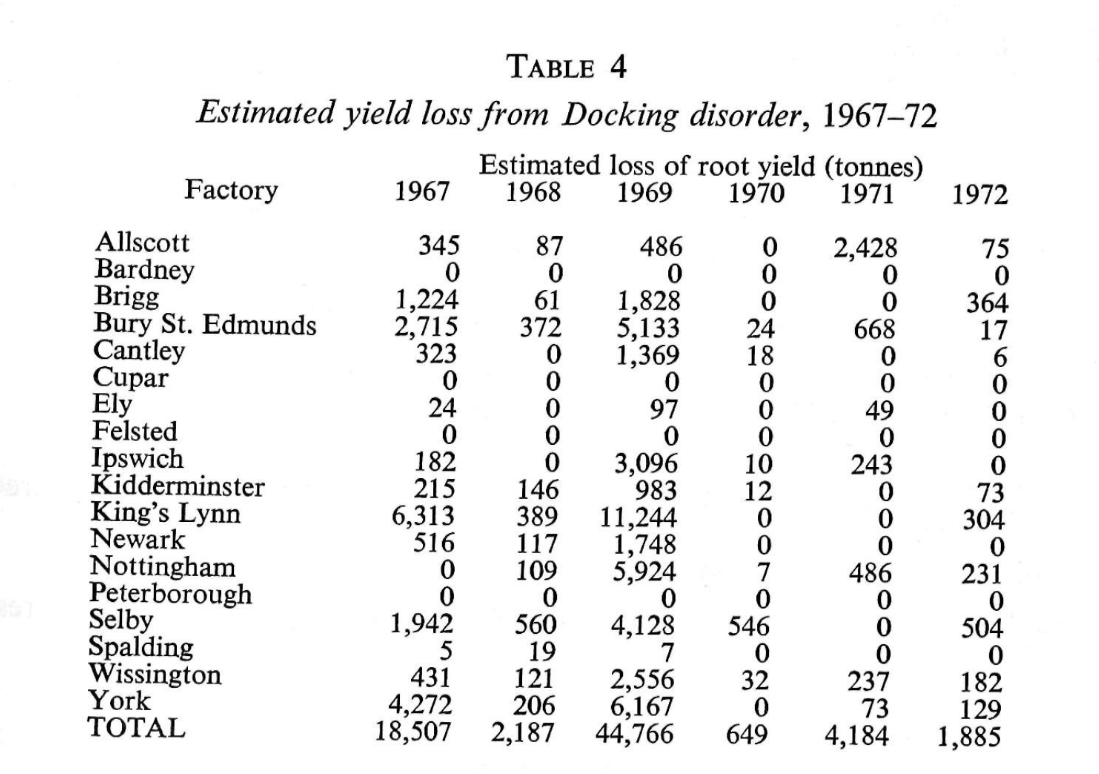
Nematodes in the family Trichodoridae are known to cause damage to economically important crops by either feeding directly on the roots (Christie and Perry, 1951) or indirectly through transmission of three viruses belonging to the tobravirus group namely: tobacco rattle virus (TRV) (Alphey and Boag, 1976), pea early browning virus (PEBV) (Hoff and Mai, 1962) and pepper ringspot virus (PRV) (Asghari *et al.*, 2018). (Gibbs and Harrison, 1963). All juvenile stages have ability to transmit viruses and virus particles are selectively absorbed in the oesophagus lining. Dissociation occurs when the nematode saliva is injected into the host, without killing of the cells for the transmission to be successful. However, juveniles lose the virus after every moult. Therefore, juveniles need to reacquire viruses to be able to transmit again while adults can retain the virus for a long period. In sugar beet in East England, *Longidorus* spp, *Trichodorus and Paratrichodorus* spp attack young seedlings causing a condition known as docking disorder, which later leads to foliage appearing to be deficient in nitrogen or magnesium (Whitehead and Hooper, 1970; Cooke, Bromilow and Nicholls, 1985; Cooke, 1989). Docking disorder is named after the parish where it was first recognised and described by Gibbs (1959). In studies done in 1963 and 1964, investigations on nematode transmitted viruses were conducted to establish whether the viruses were involved in the Docking disorder outbreaks. The findings showed that Tomato Black Ring Virus (TBRV) incidence was greater where their vectors, *Longidorus attenuatus* occurred abundantly but the symptoms were not clearly defined as typical for virus infection. The virus was also isolated from normal growing plants and also when seed transmission tests of TBRV were done. The results showed that TBRV was present in progeny of six out of eight open-pollinated, virus-infected mother plants, therefore showing that TBRV can be seed borne in sugar-beet, where the virus is transmitted to the seed by pollen. Symptoms expressed in affected plants differed where some showed a “laurel-leaf” symptom which is very different from typical mottling caused by infection from TBRV and the yellowing and stunting of these plants was unknown. When tested no TBRV was detected in these plants and therefore leading to a conclusion that yellowing and stunting outbreaks result from more than one cause or a combination of causes (Gibbs and Harrison, 1963). In studies undertaken in 1963 and 1964, investigations on nematode transmitted viruses were conducted to establish whether the viruses were involved in the Docking disorder outbreaks. Studies conducted later in 1970 by Whitehead et.al, (1970) established that nematodes in the genus *Trichodorus*, *Paratrichodorus* and *Longidorus* spp were involved in stunting of sugar beet in fields with light sandy soils in Docking. Subsequent studies have shown that trichodorids i.e. *Trichodorus primitivus* and *Paratrichodorus pachydermus* are more prevalent in fields exhibiting docking disorder (Cooke, 1989). Similar damage has also been reported from the Netherlands, where it is known as T-disease (Kuiper and Loof, 1962).

Docking disorder causes stunting of sugar beet in late May or early June. Affected beets may have symptoms of magnesium or nitrogen deficiency. The crop may recover through autumn/summer but the resulting root at harvest are often fangy/misshapen. In fields where the symptoms persisted, roots yielded 17.5 t /ha less and were more fangy than those from unaffected fields (Cooke, 1973). A yield loss of up to 50 % has been recorded as a result of the fangy root symptoms of Docking disorder (Cooke, 1989). Severity of the disorder is often worsened by environmental factors such as rainfall, previous cropping, physical conditions of the soil, rate and timing of fertiliser application/herbicides and other agricultural practices (Cooke, 1973). Root damage is mostly evident at the end of May, coinciding with higher rainfall, while the symptoms of the tops i.e. symptoms of magnesium and nitrogen deficiency are mostly visible in June (Cooke, 1973) Seedlings attacked by trichodorid nematodes may show typical damage symptoms with stubby lateral roots, which turn grey- brown and later black as they die and decay. Figure 6 below shows a fanged sugar beet root system verses a healthy root system at maturity. Any new roots formed during development are also attacked. Often the tap root stops growing or is killed and lateral roots near the surface thicken and replace it resulting in a poorly yielding misshapen (fangy) root at harvest (Whitehead and Hooper, 1970).



Figure 6:Fanged sugar beet root system (Left) verses healthy root system (Right)

**Table 1**: The proportion of the sugar-beet crop reported to be affected by Docking disorder for each factory area between 1967-1972; the areas from 1968 onwards are those reported affected in June (the month in which most Docking disorder is usually apparent). Source: Cooke, 1973.



## . **1.3 Management options for Stubby root nematodes**

## **1.3.1 Introduction**

For many decades, management strategies to minimise sugar beet yield losses caused by stubby root nematodes has been prophylactic use of pesticides i.e. use of soil fumigants. Application has been done either in autumn before sowing or as a row application shortly after drilling of sugar beet. (Cooke and Draycott, 1971). Most of the sugar beet crop at risk of docking disorder in the UK, relied on row application of granular pesticides usually aldicarb at drilling to prevent root damage by the stubby root nematodes but the expense and inconvenience of these techniques limited their use (Cooke, 1989). The annual survey by British Sugar carried out in 1985, ,suggested that there was no decrease in area of sugar beet showing symptoms resulting from nematode damage despite use of nematicides (Cooke, Bromilow and Nicholls, 1985). Nematode damage on the roots was still evident post application of carbamates, as the reversible nature of carbamate effect means that nematodes resume feeding once the active ingredient has been degraded or leached from the rhizosphere (Steele, 1977)

Effect of aldicarb phytotoxicity on decrease of seedling numbers has been reported in the past especially in dry soils following drilling, carbofuran has been similarly been associated with reducing seedling numbers when applied in direct contact with the seed.(Cooke and Holden, 1975; Maughan, Cooke and Gnanasakthy, 1984) However, consistent soil fumigation as an overall treatment in the autumn prior to planting of sugar beet increased yields in nematode affected fields (Cooke and Holden, 1975)

In general stubby root nematodes are less susceptible to soil fumigants compared to other parasitic nematodes such as sting nematodes (Grabau, Noling and Navia Gine, 2019) .Field experiments carried out for two years to evaluate the efficacy of soil fumigants ethylene dibromide (EDB), EDB + chloropicrin, and 1,3-dichloropropene (1,3-D) applied independently or in combination to Aldicarb on potato, *Solanum tuberosum* cvs. Atlantic and Sebago, for control of trichodorid nematodes and potato corky ringspot disease (CRS), showed that soil fumigation is ineffective in management of CRS in northeast Florida.(Weingartner and Shumaker, 1990). Earlier chemical management options used volatile nematicides such as DBCP, D-D or EDB which have now been banned in Europe and USA. other non-volatile nematicidal compounds such as aldicarb, carbofuran, fenamiphos and oxymyl have replaced them though they don’t act by reducing trichodorid densities but rather affecting their behaviour and host finding abilities therefore reducing virus transmission (Pelsmaeker and Coomans, 1987).

Studies have also shown that the rate of recovery by trichodorids upon use of fumigants varies considerably. In an experiment to investigate effect of fumigation on parasitic nematodes, *Paratrichodorus christie* was shown to recover more quickly compared to other parasitic nematodes (Christie and Perry, 1951) In a similar study, *P.christie* was shown to multiply more on cabbage four months post fumigation with DD or EDB when compared to unfumigated plots, numbers were however shown to be low when fumigation was done using DBCP, which had a more residual action(Rhoades, 1969).Trials done in Yorkshire, Northern England and in Norfolk, Eastern England have however obtained successful results with fumigation. In Yorkshire, 93% *Paratrichodorus anemones* were killed during winter of 1965-67 where they were fewer when compared to unfumigated plots. In Norfolk, no recovery of *Paratrichodorus teres* and *Trichodorus cylindricus* was observed after fumigation and a 99% kill was achieved during 1966-67 period. Cooke also observed that the re-establishment of *Trichodorus cylindricus* and *Paratrichodorus pachydermus* was slow upon fumigation (Cooke and Draycott, 1971) Combined use of Abamectin and azoxybitron in field experiments aimed at management of *Trichodorus obstutus* on zoysiagrass, showed that the root weight of treated plants increased by 0.50 and 0.81 g respectively compared to untreated controls. (Shaver, Agudelo and Martin, 2016).The British beet research organisation (BBRO) recommends assessment of SRN densities before application of any nematicide. The set threshold for management measures i.e. nematicide application is 1000 trichodorid per 1 litre of soil. However, Vydate (Oxymyl), which has been the nematicide applied by the sugar beet growers in the recent past, was banned in UK in December 2020, leaving growers with no chemistry for the management of free-living parasitic nematodes (FLN). Discussions have been made on whether other nematicides used in other crops such as nemathorin (Fosthiazate), Mocap (ethoprophos) or NEMGuard (garlic extract) can been used in sugar beet to manage FLN. However, none of these products have been registered for sugar beet and BBRO also expresses doubts on whether these products will be registered soon as past tests with nemathorin on sugar beet indicated that crop safety was not sufficient to justify its development in the crop (Stevens, 2015) As the pressure to develop other active ingredients seems hard to execute., other cultural and crop management strategies need to be evaluated for future recommendations to sugar beet growers (Stevens, 2015)

### **1.3.2 Use of cover crops for the suppression of plant parasitic nematodes (PPN)**

Cover crops are plants planted at intervals within a normal cropping rotation with the main crop to improve soil structure, soil fertility and water infiltration (Fourie et al., 2016). The continuous improvement of soil organic matter promotes populations of free-living nematodes which play a key role in nutrient cycling ( Wang et al., 2008). Cover crops left on the soil surface are also utilized as mulch and provide additional benefits such as slow release of nutrients from the residues, and nematicidal compounds associated with them are also released slowly over long periods of time. Use of cover crops may provide a potential nematode management option as certain cover crops can reduce nematode populations by either 1) acting as resistant hosts, poor hosts or non-hosts, 2) producing allelochemicals that are toxic or inhibitory, 3) providing an ecological niche for antagonistic flora and fauna and 4) trapping the nematode (Wang et al., 2002). Some cover crops produce secondary metabolites, which are products released during plant growth and development. The secondary metabolites play a key role in defense against pathogens and pests. The main secondary metabolites produced are alkaloids, flavonoids, monoterpenoids, diterpenoids, and polyphenols and some have nematicidal properties Alkaloids, monoterpenoids, saponins, pentacyclic, triglycerides, sesquiterpenes, steroids, diterpenes, flavonoids and glucosinolates have been shown to exhibit nematicidal activity (Chitwood, 2002). These secondary metabolites have been further exploited for development of biopesticides for nematode management (Renco, Sasanelli and Maistrello, 2014). Compounds from widely used cover crops such as polythienyls and polyacetylenes from family Asteraceae, isothiocyanates from Brassicaceae, alkaloids from Leguminosae and glucosides from Poaceae.2-dehydropyrrolizidine alkaloids (PAs), particularly associated with plants belonging to the families Asteraceae, Boraginaceae and Fabaceae have also been shown to suppress nematodes (Thoden, Boppré and Hallmann, 2009).

Cover crops can promote antagonistic flora and fauna and these includes some nematode antagonists. Nematode antagonists refer to parasites, predators or microorganisms that either repel, inhibit, compete or kill plant parasitic nematodes. Some cover crops enhance antagonists such as fungal egg parasites, nematophagous fungi, nematode trapping fungi, endoparasitic fungi, plant health promoting rhizobacteria and obligate bacterial parasites. Several hypotheses on how cover crops enhance antagonistic microrganisms have been described. For example, Linford (1937) speculated that incorporation of biomass from cover crops promotes proliferation of bacteria during decomposition of the organic material, which become a source of food for microbiovorous nematodes and in turn become a food base for nematophagous fungi (Van den Boogert et al, 1994b). A study evaluating incorporation of *Crotolaria juncea* (Sunn Hemp) demonstrated that the reproduction of the bacterivorous nematode *Acrobeloides bodenheimeri* which is a prey to nematophagous fungi *Hirsutella rhossilensis* was increased (Venette, Mostafa and Ferris, 1997) *.*

Incorporation of organic material has also been shown to promote mycostatis which is a scenario where the inability of parasitic fungus to germinate facilitates proliferation of antagonistic fungus which in turn suppresses parasitic nematodes (Stirling, 1988). Leguminous crops have been shown to promote proliferation of nematophagous fungi compared to other crops. Microplots amended with Alfalfa stimulated two key nematode trapping fungi *Arthrobotrys dactiloydes* and *Dactylellina elipsospora* (Van den Boogert et al.,1994a)*.* In the presence of nematodes or homogenates of nematodes, traps i.e. either constricting rings, adhesive knobs or adhesive branches of the fungus are induced (Nordbring-Hertz, 1997). Pea crops have also been shown to stimulate nematode trapping fungi to a greater degree than mustard or barley; where the conidial traps of the nematode trapping fungus (*Athrobotrys oligospora*) were more abundant in the pea rhizosphere than in fallow/root free soil (Persmark and Janson, 1997). Similarly, the suppression of *Rotylenchus reniformis* following soil amendment with *C. juncea* leaves was correlated with increases in nematode trapping fungi, fungal egg parasites and bacterivorous nematodes.The nematode trapping fungus isolated upon soil amendment with C.juncea leaves were *Monocosporium ellipso- spora* and *Arthrobotrys dactyloides*, which in other studies have been shown to suppress *Meloidogyne javanica* and have been formulated for use as biocontrol agents *(Wang et.al.,2001). C.juncea* in these studies was shown to be a poor host to *R. reniformis,* have allelopathic effects when leaves were incorporated in the soil and promote antagonistic nematode trapping fungus. *Raphanus sativus* is a cover crop that has been shown to have multiple attributes where it can serve as a cover crop, trap crop, cash crop or a biofumigant crop. As a trap crop to *Heterodera schachtii*, it allows infection by the nematode but inhibits completion of the life cycle while as a biofumigant crop, it produces isothiocyanates from hydrolysis of glucosinolates, that suppresses soil borne pests (Aydınlı & Mennan, 2018). *Eruca sativa* (Rocket or Arugula) has also been used as a trap crop in the management of the Northern Root Knot Nematode (*Meloidogyne hapla*); the nematodes are attracted to the roots but are unable to reproduce , which lowers the population densities (Aydınlı and Mennan, 2018)

### **1.3.3 The use of brassicaceous crops for the PPN management**

Members of the brassica family are used for management of plant parasitic nematodes (PPNs) either as green manures or as seed meals. Seed meals consist of the residual products of brassica seeds after oil extraction and are spread and incorporated in the soil as pellets. Green manuring involves planting of the brassicas and incorporating them at the flowering stage (Lord et al., 2011; Ngala et al., 2014b; Zasada et al., 2009). Maceration and incorporation of brassica residues into the soil is known as biofumigation. This chemical process involves breaking down of the tissues to produce a range of bioactive compounds including isothiocyanates (ITCs) within the soil (Lord *et al.*, 2011; Ntalli and Caboni, 2017). The term biofumigation was first used to refer to the suppression of soil borne pathogens, weeds, and pests via hydrolysis of incorporated brassica residues (Kirkegaard *et al.*, 1993) Major brassicas with biofumigant properties include *B. oleracea* (broccoli, cabbage, cauliflower, Brussels sprouts, kale), *B. napus* (rapeseed and canola), *B. rapa* (turnip), *Raphanus sativus* (radish), *B. campestris* (field mustard), *B. juncea* (Indian mustard), *Sinapis alba* (white/yellow mustard), *B. nigra* (black mustard), *B. carinata* (Ethiopian mustard), *Eruca sativa* (salad rocket) (Dutta, Khan and Phani, 2019).

The process of biofumigation is explained by the fact that Brassicas contain a class of thioglucoside secondary metabolites known as glucosinolates (GSLs). Glucosinolates were first described in the 17th century in research investigating the chemicals responsible for the bitter taste in mustards (Challenger, 1960). Glucosinolates are limited to the order Capparale which includes the families Brassicaceae, Capparaceae, Resedaceaae and Moringaceaae (Brown *et al.*, 2003). Sinigrin (2-propenyl or allyl glucosinolates) and sinalbin (4-hydroxybenzyl glucosinolates) were the first GSL to be isolated from *Brassica nigra* (Black mustard) and *Sinapis alba* (white mustard) respectively (Fahey, Zalcmann and Talalay, 2001). GSLs are sulphur containing metabolites, stored in the cell vacuole. Chemically, they exist as β-thioglucoside from amino acids and are categorized based on the structure of their side chain (R). GSLs occur in different quantities and have different profiles both quantitatively and qualitatively within the family brassica, in different cultivars and even species grown in the same environment (Bellostas, Sørensen and Sørensen, 2004). For instance, the rapeseed variety Hyola 401 contains lower GSL content compared to the variety Dwarf Essex; Indian mustard cultivar has higher levels of GSL compared to Ida Gold variety of white mustard (Dutta, Khan and Phani, 2019).

The quantities and type of GSLs produced varies between plant organs, genetic makeup of the species, developmental stages, and exposure to environmental factors such as soil nutrients (nitrogen and sulphur), seasonal variations, or drought. For instance, variation in GSL concentration was observed in similar broccoli genotype grown at different seasons and under distinct agricultural practices (Bhandari, Su Jo and Gu Lee, 2015). A study investigating the distribution pattern of GSLs in different parts of Brassica crops, showed that GSLs were highly concentrated in the seeds, followed by the sprouts, roots and shoots. The aromatic GSLs were highest in roots while the aliphatic GSLs were concentrated in seeds and the indole GSLs were more concentrated in the roots or shoots of tissues of most brassicas (Bhandari, Su Jo and Gu Lee, 2015).

The basic structure of GSLs is similar although variations occur in the R-group which is related to their biosynthesis (Schonhof, Krumbein and Brückner, 2004). There are more than 130 GSL that have been identified that are structurally different and are divided into different classes based on structure of amino acid derived side chain (R) (Buskov *et al.*, 2002). They are categorized into three classes namely: Aliphatic, aromatic and indolyl/indole, with sinigrin usually being the predominant GSL being identified from Brassicaceae plants (Kruger et al., 2013). Aliphatic glucosinolates are known to be derivatives of 5 methionine, aromatic glucosinolates from tyrosine or phenylalanine, while indole 6 glucosinolates are derivatives from tryptophan (Schonhof, Krumbein and Brückner, 2004)and the latterdo not produce isothiocyanates (ITCs) and are therefore not relevant in biofumigation. Table 3 below illustrates the nomenclature, source and structure of different glucosinolates

Table 2: Glucosinolates nomenclature, source, structure and acronyms (Wathelet et.al., 2004)

|  |  |  |  |
| --- | --- | --- | --- |
| **Category 1: Aliphatic and Arylaliphatic** | | | |
| **Glucosinolate** | **Source** | **Side chain** | **Acronym** |
| Sinalbin | *Sinapis alba* | 4-hydroxybenzyl | SNB |
| Sinigrin | *Brassica juncea* | 2-propenyl or allyl | SIN |
| Gluconapin | *Brassica rapa* | 3-butenyl | GNA |
| Glucocapparin | *Capparis spinosa* | Methyl | GCA |
| Glucobarbarin | *Barbarea vulgaris* | (R)-2-hydroxy-2-phenelethyl | GBB |
| Gluconarsturtin | *Barbarea verna* | 2-phenylethyl | GST |
| Glucolimnanthin | *Limnanthes sativum* | 3-methoxybenzyl GLI |  |
| Glucotropaeolin | *Lepidium sativum* | Benzyl | GTL |
| Glucobrassicanapin | *Brassica rapa* | 4-pentenyl |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Category 2: Hydoxylated aliphatic** | | | |
| Protogoitrin | *Brassica napus* | (R)-2-hydroxy-3-butenyl | PRO |
| Glucosisymbrin | *Sisymbrium loesilii* | 2-hydroxy-1-methylethyl | GSY |
| Glucoringiin | *Conringia orientalis* | 2-hydroxy-2-methylpropyl | GCN |
| Glucoceomin | *Conringia orientalis* | 2-hydroxy-2-methylbutyl | GCL |
| Epi-progoitrin | *Crambe abyssinica* | 2-hydroxy-3-butenyl | ePRO |
| Gluconapoleiferin |  | (R)-2-hydroxy-3-pentenyl | GNL |

|  |  |  |  |
| --- | --- | --- | --- |
| **Category 3: thiofunctionalised** | | | |
| Glucobervirin | *Thlaspi sempevirens* | 3-methiopropyl | GIV |
| Glucoiberin | *Iberis amara* | 3-methylsulfinylpropyl | GIB |
| Glucocheirolin | *Cheirantus annus* | 3-methylsulfonylpropyl | GCH |
| Glucoerucin | *Eruca sativa* | 4-methiobutyl | GER |
| Glucoraphanin | *Broccoli* | 4-methylsulfinylbutyl | GRA |
| Glucoraphasatin | *Raphanus sativus* | 4-methylthio-3-butenyl | GRH |
| Glucoraphenin | *Raphanus sativus* | 4-methylsufunyl-3-butenyl | GRE |
| Glucoalyssin |  | 5-methylsufunylpentyl | GAL |
|  |  |  |  |
|  | | | |
| **Category 4: Indole type** | | | |
| Glucobrassicin | *Isatis tinctoria* | 3-indolylemethyl | GBS |
| 4-OH-Glucobrassicin |  | 4-hydroxy-3- indolylemethyl | 4-OHGBS |
| 4-OMe Glucobrassicin |  | 4-methoxy-3-indolylmethyl | 4-OMeGBS |
| Neo-glucobrassicin |  | 1-methoxy-3-indolylmethyl | neo-GBS |

#### **Degradation of glucosinolates**

Physical damage of brassica plant tissues such as maceration, slow freezing, thawing, chopping or chewing, causes release of the glucosinolates which are localised in vacuoles by cell organelles into the cell cytoplasm where it is comes into contact with endogenous thioglucosidases (myrosinases), located in the cell cytoplasm of separate cells (myrosin cells) and is hydrolysed (Brown *et al.*, 2003).The hydrolysis results to conversion of the compounds to corresponding aglycons which then decompose to release of bioactive compounds such as nitriles, thiocyanates, and isothiocyanates depending on the R-group and prevailing chemical conditions in a process known as GLS-MYR system (Dutta et al., 2019a; Ngala et al., 2014;Wathelet, 2004). Brassicas additionally produce other toxic sulphur containing hydrolysis products such as dimethyl sulphide, methyl sulphide, dimethyl disulphide, carbon disulphide, methaneiol etc., which may contribute to the biofumigation process Dutta et al., 2019). Glucosinolates are also occasionally hydrolysed from enzyme myrosinase produced *in situ* by soil microbes. Isothiocyanates (ITC) are the most toxic glucosinolates catabolites and are attributed to the biocidal activity of brassica green manures (Dutta et al., 2019). Some studies have suggested the possible reactions that occur between the nematode pest and the ITCs, one of them is reaction of the active sites of the ITC with the nucleophiles of the nematode, mainly thiols and amine groups of certain enzymes making them alkylated. In other cases, the ITC have been shown to induce oxidative DNA damage and also affect the motility of the nematode by impairing its host finding ability. In an isolated study, it was observed that dorsal pharyngeal gland nucleus in *Globodera rostochiensis* reduced upon exposure to ITC hence ultimately reducing the nematode parasitism (Dutta et al., 2019). The non-volatile residues produced by biofumigant crops also improve the soil organic matter, recycle nutrients hence contributing to good soil quality that gradually build management of soil borne pathogens. Figure 10 below shows the release of ITC upon hydrolysis of glucosinolates

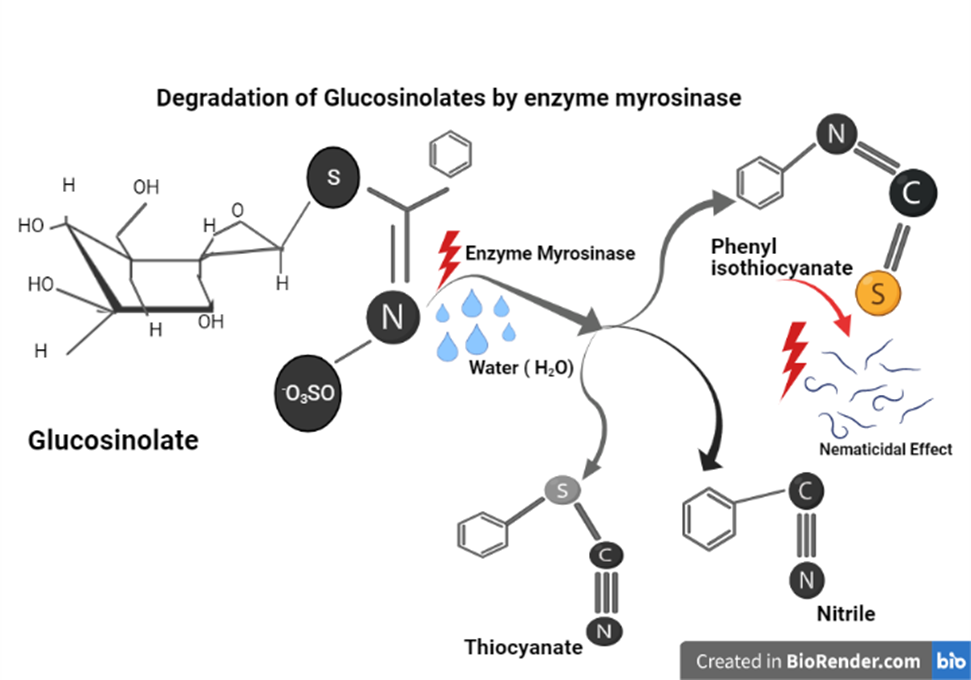


Figure 7: Hydrolysis of glucosinolates into isothiocyanates, thiocyanate and nitriles by enzyme myrosinase.

#### **b) Toxicity of isothiocyanates**

The ITC derivatives differ in their level of toxicity among and within the different Brassica species (Zasada and Ferris, 2003). Their effectiveness differs depending on the type and structure with the most effective being 2-phenethyl-isothiocyanate (PEITC).(Pinto, Rosa and Santos, 1998; Matthiessen and Kirkegaard, 2006). This difference in toxicity can also be partly explained by the different biosynthetic pathways that are influenced by both genetic and environmental factors (Mithen, 2001; Li & Quiros, 2003; 4 Windsor *et al*., 2005). The toxicity of aromatic ITC ‘s *to Tetrahymena pyriformis* have been correlated to reaction with cysteine residues of glutathione which has a role in respiration (Schultz, Yarbrough and Woldemeskel, 2005).Toxicity of ITC to nematodes is also known to be influenced by ITC-lipid solubility, ITC volatility and ITC hydrophobicity. Volatile ITC e.g. 2-propenyl are in gaseous in form and are capable of dispersing evenly under suitable conditions and effectively interact with the target organism. Lipid soluble ITC’s e.g. 2-phenethyl are able to penetrate the nematode cuticle and permeate phospholipid membranes hence interacting with intercellular functions that kill the organism. (Sarwar *et al.*, 1998) However, the toxicity of the ITCs under field conditions are influenced by many other factors related to agronomic practices, soil factors and prevailing climatic conditions. Therefore, achievement of effectiveness involves manipulation of these factors in a pest and disease management (Ahuja *et al*., 2010b). For instance, high organic matter content was found to lead to sorption of Methyl ITC, which hence reduced the available ITC in the soil for pathogen suppression (Smelt & Leistra 1974). High levels of organic carbon in the soil has also been associated with decrease in 2-propenyl ITC (Borek *et al*., 1995a). Similar observation has also been made on methyl ITC decrease in soils with high organic carbon. A possible explanation for the decrease may have been the reaction of the ITCs with nucleophilic groups such as phenols, amines, alcohols, carboxylic acids, 7 and thiols contained in soil organic matter. The toxicity of the ITCs has also been shown in a study by Buskov et.al (2002), to be a related to type of glucosinolates in the brassica species used, where out of 13 glucosinolates tested, a high mortality of PCN juveniles was recorded after exposure to brassica extracts phenethyl- and benzyl glucosinolates which are both capable of producing isothiocyanates upon hydrolysis. Similarly, another study evaluating efficacy of ITC produced by different plants recorded that *B. vulgaris* and *Moricandia moricandioides* lacked efficacy against *Globodera pallida, due to* the fact that they contain indole-glucosinolates which are unable to produce stable ITCs*.* (Halkier and Gershenzon, 2006)*.*

##### **In-vitro studies on nematicidal activity of ITC**

*In-vitro* studies have been conducted under controlled conditions to determine the nematicidal activity of brassica volatiles exposure to different stages of plant parasitic nematodes. Studies have been conducted using both commercially available pure isothiocyanates and natural extracts either derived from leaf, shoot and root extracts and macerates of different brassica crops. In these assays, nematodes were exposed to isothiocyanates or their hydrolysis products at different concentrations and exposure time. The ITCs of rapeseed glucosinolates, i.e. gluconapin, glucotropeolin and dehydroerucin caused mortality of almost all juveniles of *Heterodera schactii* at a concentration of 0.5% after 48 hours while mortality of juveniles was recorded only after 24 hrs exposure to ITCs from sinigrin at similar concentration. Further tests to investigate the toxicity of sinigrin ITCs showed that even at concentration of 0.05%, of crude extracts obtained from whole seed of *Brassica carinata* caused 100% juvenile mortality of *Heteroders schactii* after 48hrs exposure time.No nematicidal activity was, however, recorded from the hydrolysis products of sinalbin and glucoraphanin (Lazzeri, Tacconi and Palmieri, 1993). Similar observations were recorded in an experiment where the effects of eight glucosinolates were tested: Prop-2-enyl-, but-3-enyl-, (R)-4-methylsulfinylbut-3-enyl-, benzyl-, phenethyl-,4-hydroxybenzyl-, (2S)-2-hydroxybut-3-enyl-, and (2R)-2-hydroxy-2-phenylethylglucosinolate and their hydrolysis products were tested against juveniles of *Globodera rostochiensis.* The glucosinolates were used at three concentrations, 0.05, 0.3, and 1.0 mg/mL, in the presence or absence of the enzyme myrosinase. Results showed that intact glucosinolates had no effect in the absence of myrosinase enzyme. However, 1 mg/mL phenethyl glucosinolates at pH 6.5, with the addition of myrosinase, caused 100% mortality of the juveniles following a 16 hours exposure time (Buskov *et al.*, 2002). 2-propenyl isothiocyanate, at a concentration of 0.002%, was also shown to exhibit high toxicity on eggs of *Globodera pallida*, where the hatching ability was decreased by 50% after 2hrs exposure time (Brolsma *et al.*, 2014).

A sand column assay was carried out to test the effect of leaf extracts from twenty-two brassica accessions on the motility of juveniles of *Globodera pallida* . Leaves were obtained from 8-week old brassica plants, they were then flash frozen in liquid nitrogen and ground into powder, which was dissolved in sterile water at the beginning of each assay. The juvenile motility was measured by checking their movement down a sand column. Results from the study indicated that the, leaf extracts of especially the brassica varieties *Raphanus sativus* cv. Weed check, *N. officinale* cv. Cress, and *Brassica juncea* cv. Nemfix were very potent where they significantly inhibited motility of *Globodera pallida* juveniles, by 97, 93, and 89% inhibition, respectively (Lord *et al.*, 2011).

In another study, silica sand in polyvinyl tubes was used to determine the lethal concentration of commercially available isothiocyanates i.e. Allyl, benzyl, butyl, ethyl, phenyl, 2- phenylethyl and 4-methyl-sulfinyl(butyl) against *Tylenchulus semipenetrans* and *Meloidogyne javanica.* The tubes were 25°C for 48 h.The findings indicated that benzyl and 2-phenylethyl ITCs, with the highest molecular weights, were the most toxic ITCs. The LC90 values were 0.01 and 0.03 μmol/ml for 2-phenylethyl isothiocyanate and 0.01 and 0.06 μmol/ml for benzyl isothiocyanate for *T. semipenetrans* and *M. javanica*, respectively (Zasada and Ferris, 2003).

Effect of commercially available pure isothiocyanates, namely ethyl isothiocyanate, propyl isothiocyanate, isopropyl isothiocyanate, butyl isothiocyanate, isoamylene isothiocyanate, acryloyl isothiocyanate, isovaleryl isothiocyanate, phenyl isothiocyanate, benzyl thiocyanate, benzyl isothiocyanate, 1-phenylethyl isothiocyanate, 2-phenylethyl isothiocyanate and allyl isothiocyanate, were tested against *Meloidogyne javanica and* compared to metam sodium in an *in vitro* experiment. A vessel containing the ITCs was placed at the bottom of desiccator bottles and nematodes placed at the top of the desiccator inside a multi well plate*.* When exposed for three days to allyl, acroloyl and ethyl ITCs, the juveniles became irreversibly immobile at LC50 values of 2.76, 2.53 and 3.05 mg mL-1 , respectively (Wu *et al.*, 2011).

In an experiment investigating the effect of volatile organic compounds on *Meloidogyne incognita*, the macerated tissue of broccoli shoots (*Brassica oleracea* var. Itálica) and sunflower seeds (*Helianthus annus)* were examined*.* Volatile organic compounds (VOC) were shown to reduce the infectivity, mobility, and reproduction of *Meloidogyne incognita* juveniles. The mobility, infectivity and reproduction of juveniles was also reduced when they were placed in water exposed to broccoli. This is because sulphurated VOC were found in the water exposed to the broccoli macerates and were attributed to the nematotoxic effects to the juveniles. The study also showed that sunflower produces toxic volatile organic compounds that have potential use in biofumigation against *Meloidogyne incognita.* (Carlos *et al.*, 2018). Volatile compounds obtained from seeds of *Brassica juncea*, *Brassica napus* and *Sinapis alba* similarlyindicated that they had a nematicidal activity when exposed to *Paratylenchus* spp and populations of *Aphelenchoides compositola* parasitizing white button mushroom (Kowalska and Smolinska, 2001).

##### **II. Invitro studies on nematicidal activity of non-brassica crops**

*In vitro* studies with compounds derived from non-brassica crops have also registered varied nematicidal activities. Tannins are secondary metabolites and classified as plant polyphenols with a high affinity to proteins and polysaccharides. Their physical and chemical profile is variable depending on the season of production, organ of the plant and the plant species (Maistrello, Vaccari and Sasanelli, 2010). Tannins are known to act as defence compounds, protecting the plant from herbivory (Maistrello, Vaccari and Sasanelli, 2010; Renco, Sasanelli and Maistrello, 2014). A reduction in *Longidorus elongatus* was observed when infested soil was mixed with powdered tannins from Mimosa (*Acacia mollirrima* Wild.) and quebracho (*Schinopsis lorentzii*). The nematicidal activity was attributed to the phenolic acids and their derivatives that are contained in tannins (Taylor and Murant, 1966). Aqueous tannin solutions of chestnut (*Castanea sativa* L.) were also shown to be effective against *Meloidogyne javanica*.

Alkaloids, sanguinarine, chelerytherine and allocryptopine, extracted from *Macleaya cordata,* also known as plume poppy or *Bocconia cordata*, a perennial herb of the family Papaveraceae, were tested for their nematicidal activity against *Bursaphelenchus xylophilus*, *Caenorhabditis elegans* and *Meloidogyne incognita* in an *in vitro study.* The alkaloids exhibited nematicidal activity at LC50 28.52, 34.50 and 37.45 µg/ml, respectively, against *B. xylophilus*; 22.78, 40.25 and 38.90 µg/ml, respectively, against *C. elegans*; and 67.52, 61.00 and 76.56 µg/ml, respectively, against *M. incognita* at 24 h (Wang *et al.*, 2012). The alkaloid, 1,2- dehydropyrrolizidine (PAs), extracted from *Chromolaena odorata* and the invasive weeds, *Crotalaria* spp. or *Ageratum* spp. had nematicidal, ovicidal and repellent effects on free/living and plant parasitic nematodes. A repellent effect was observed for the *Rhabiditis* spp but not recorded in *Meloidogyne incognita* in the study, showing the variability in susceptibility of the different nematodes to the 1,2- dehydropyrrolizidine (Thoden, Boppré and Hallmann, 2009). An *in vitro* experiment using leaf and stem extracts of Bean (*Phaseolus vulgaris* L.) and leaf extracts of tobacco (*Nicotiana tabacum* L.) showed that the extracts were able to slow down the movement of *Hoplolaimus* spp. and *Tylenchorynchus dubius* (Bütschli) Filipjev at 1hour exposure time. The nematicidal activity was still observed when the extracts were added to the soil showing practicality in real field situations (Miller, Turner and Tomlinson, 1973).

##### **c) Potential of biofumigation under field conditions**

The potential of biofumigant brassica crops has also been extensively studied on different target nematodes under field conditions (Table 2). The effects have been inconsistent with some studies recording high suppression upon incorporation of brassica residues (Lord et al., 2011) and in some no effect to the target species (Vervoort et al., 2014). To be effective, brassica green manures need to effectively release ITCs for soil fumigation under suitable conditions. A blend of high soil moisture content, thorough pulverization in the incorporation process and high plant biomass are vital in release of ITCs for biofumigation (Matthiessen and Kirkegaard, 2006) In the incorporation process, the strategy of incorporation and proper timing is essential. A study by Matthiessen, Warton and Shackleton (2004), showed that approximately 100nmolg-1 .ITC soil concentrationwas achieved following thorough pulverization and irrigation after incorporation of mustard. Similarly, successful biofumigation was achieved by combined effect of using Indian mustard *Brassica juncea* and intense mechanical tillage during green manuring which effectively decreased the population densities of *Trichodorus* spp and *Tylenchorynchus* spp rather than ITCs of *B. juncea* alone (Vervoort et al., 2014;Dutta et al., 2019b). On the contrary, a study by Gardiner *et al.*, (1999), recorded a different concentration of 1nmolg-1 following plough down of winter rapeseed which is below the recommended rate of 260 pounds per acre of Methyl ITC required for effective suppression of pests and pathogens. Nevertheless, release of low concentrations over a long period of time can ultimately lead to suppression of pests and pathogens (Mattner *et al.*, 2008) This is especially in the case of partial biofumigation, where brassica crops are grown but not incorporated. GSLs released from the plant roots during its growth period are slowly hydrolysed by enzymes released by soil microbes,(Ngala, Woods and Back, 2015) This strategy is beneficial to winter hardy crops biofumigants such as *Raphanus sativus* which was found to have large root biomass hence release of good amounts of GSLs which effectively in suppressed of *Globodera pallida* (Ngala, Woods and Back, 2015).Timely incorporation of brassica materials is another key factor in maximising efficacy of biofumigants. Studies by Mattner *et al*., (2008) indicated that higher efficacy was obtained from maceration and incorporation of mature plants as compared to immarture plants. Similarly, the suppression of *Rhizoctonia fragarie* using *Brassica rapa* and *Brassica napus* green manures was greatest when maceration and incorporation was done at anthesis compared to maceration at establishment stage (Mattner *et al.*, 2008)￼This is because the GSL concentration is highest/at peak during mid-flowering and this timing should be closely monitored (Ngala *et al.*, 2014)￼ Plant biomass which is another contributor to efficacy, can vary greatly between and within various brassicas species, For instance 70t ha-1 fresh weight biomass was recorded in *Brassica juncea* which was two times higher than *Eruca sativa* grown under similar field conditions during mid-flowering stage ( Ngala *et al.*, 2014 ;Watts *et al.*, 2014

High glucosinolates brassica crops can achieve more than 40 μmol GSL g-1 dry-weight tissue (Kirkegaard & Sarwar, 1998; Lord *et al*., 2011). Low levels of brassicaceous residues incorporated (20kg/ha) were not effective in suppression of *Meloidogyne incognita* as compared to 60kg/ha which effectively reduced infection and damage of *Meloidogyne incognita* in *Vigna subterranean (Fourie,2015)*.The high amount of root biomass produced by *Raphanus sativus* (oilseed radish) was linked to its effective partial biofumigation in reducing the viability of encysted eggs of *Globodera pallida, the* high root biomass which was linked to production of high concentration of glucosinolates hence toxic isothiocyanates (Ngala et al., 2014). However, It is also important to consider suitable planting season as seasonal variations in biomass produced exist between summer and winter sown brassicas.(Booth, Walker and Griffiths, 1991; Sarwar *et al.*, 1998; Price *et al.*, 2005). In summer, glucosinolates concentration of upto 100 μmol GSL g-1 dry-weight tissue has been recorded in *Brassica juncea* cv. ISCI 99, at mid-flowering stage under field conditions (Ngala *et al*., 2014). The high concentration in summer, has been attributed to light intensity, long day length and temperatures which are higher in summer (Engelen-Eigles *et al.*, 2006)

The long day length and light intensity ensures high photosynthesis hence accumulation of glucose which is an important integral of GSL as well as contributing to high biomass (Agerbirk and Olsen, 2012). Summer grown brassicas for that reason have been shown to be more effective in pest and disease suppression as compared to winter grown brassicas. Nutrients such as sulphur and nitrogen are also very paramount in field grown biofumigants and are important elements in the biosynthesis process of GSL which greatly influences the GSL concentration. Nitrogen also plays a vital role in protein biosynthesis, cation which influences the biomass produced by the biofumigant. Recommended field application rate for Nitrogen is 60-100 kg ha-1  (Lazzeri *et al.*, 2004) while sulphur is applied as sulphate at a ratio of 5:1 (Pers. Comm. Dr Matthew Back: Reader in Nematology at Harper Adams University investigating nutrient applications to biofumigant crops for AHDB Potatoes).

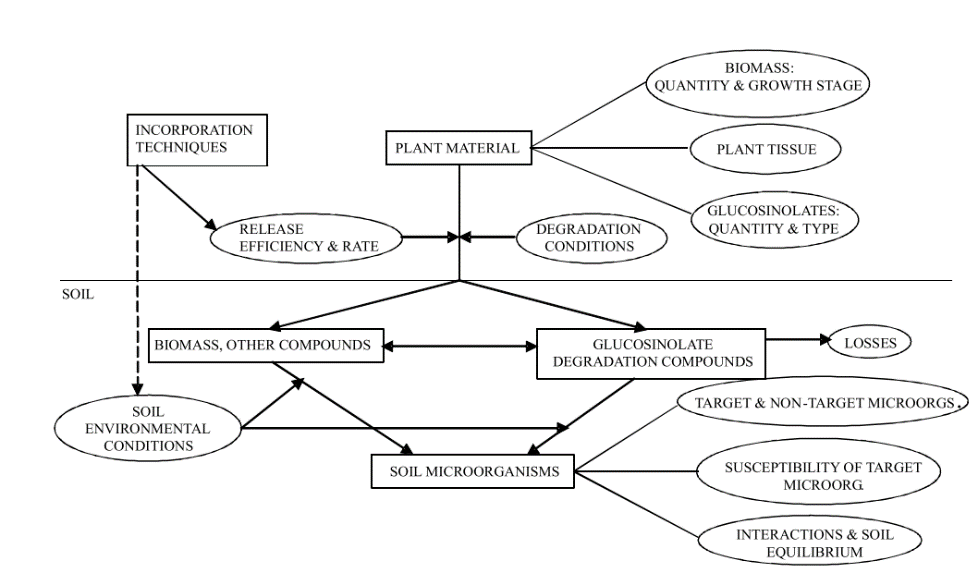
The content and profile of glucosinolates in the plant tissue is also key as it has been linked to effective suppression of pests and pathogens, this because the ITCs produced are dependent on the GSL content and profile (Fourie *et al.*, 2017; Dutta, Khan and Phani, 2019).Production of high levels of aliphatic ITCs by some brassicas has been shown to give greatest suppression to soil borne pathogens (Matthiessen and Kirkegaard, 2006). Other compounds released during decomposition of brassica materials have also been shown to contribute to the process of biofumigation. Compounds such as methyl sulphide, dimethyl sulphide, dimethyl disulphide, carbon disulphide and methanethiol (Lewis and Papavizas, 1971)These compounds have low toxicity compared to allyl ITCs but are released over a longer period of time hence their suggested contribution in biofumigation process (Walker, Morell and Foster, 1937; Virtanen and Wahlross, 1965; Lewis and Papavizas, 1971). Resistance trait in brassicas has also been shown to contribute to efficacy of biofumigants. In Eastern England, field studies were conducted to evaluate the efficacy of resistant lines of oil radish (*Raphanus sativus*) and mustards (*Sinapis alba*) as green manure cover crops in suppression of beet cyst nematodes (BCN) in. Class 1 radish (with ≥ 90% resistance level) and Class 2 mustard (with ≥ 70-90% resistance level) significantly reduced the levels of BCN density when compared to the fallow control. 30-40% reduction of BCN densities was recorded, and this effect was attributed to the good growth habits of the brassicas which was measured by Normalized Difference Vegetation Index (NDVI) and their ability to stimulate BCN hatching.(Wright *et al.*, 2019). Figure 9 below shows the factors affecting the outcome of biofumigation and Table 2 shows studies conducted on efficacy of green manures on different nematode families, genera and species under field conditions.

Figure 8: Summary of different factors affecting the outcome of Biofumigation (Bellostas, Sørensen and Sørensen, 2004))

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Table 3:Effects of Biofumigation on plant parasitic nematode populations using different Brassicaceae species (Fourie *et al.*, 2016) | | | | | | |
| **Cover crop** | **Follow -up crop** | **Type of amendment** | **Nematode spp** | **Reduction %** | **Country** | **Reference** |
| *Brassica campestris* | *Solanum tuberosum* | Green manure | *Meloidogyne chitwoodi, Pratylenchus neglectus* | 48% reduction in *M. chitwoodi* juveniles and 54% reduction *P. neglectus* | USA | Al-rehiayan et al (1999) |
| *Brassica campestris* | *Vitis vinifera* | Green manure | *Meloidogyne javanica* | 61-73% reduction in egg production | Australia | (McLeod and Steel, 1999) |
| *Brassica carinata* | N/A | Green manure | *Pratylenchus neglectus* | 0-65% reduction | Australia | (Potter, Davies and Rathjen, 1998) |
| *Brassica juncea* | *Vitis vinifera* | Green manure | *Meloidogyne javanica, Criconemoides xenoplax* | 51% reduction in M.javanica and no effect on C.xenoplax | South Africa | Kruger et al.,2015, Kruger (2013) |
| *Brassica juncea* | *Solanum tuberosum* | Green manure | Globodera pallida | Significant reduction in viable encysted eggs | United Kingdom | Ngala et al., (2014) |
| Brassica juncea | N/A | Green manure | *Trichodorus* spp, *Tylenchorynchus* spp | No significant reduction in population levels | Germany | Vervoort et al., (2014) |
| **Cover crop** | **Follow -up crop** | **Type of amendment** | **Nematode spp** | **Reduction %** | **Country** | **Reference** |
| *Brassica juncea* | *Solanum lycopersicum* | Green manure | *Meloidogyne incognita* | 14% reduction in galling | India | (Randhawa and Sharma, 2008) |
| Cover crop | Follow -up crop | Type of amendment | Nematode spp | Reduction % | Country | Reference |
| *Brassica juncea* | N/A | Green manure | *Pratylenchus neglectus* | 6-68% reduction in population levels | Australia | (Potter, Davies and Rathjen, 1998) |
| *Brassica napus* | *Vitis vinifera* | Green manure | *Meloidogyne javanica, Criconemoides xenoplax* | Reduced by 14 and 8% for M.javanica and C.xenoplax respectively. | South Africa | (Kruger, Fourie and Malan, 2015) |
| *Brassica napus* | *Vitis vinifera* | Green manure | *Pratylenchus neglectus* | 0-57% reduction in population levels | Australia | Potter et al.,1998 |
| *Raphanus sativus* | *N/A* | Green manure | *Globodera pallida* | 50% reduction in population levels | USA | (Riga, Wilson and Dossey, 2010) |
| *Raphanus sativus* | *N/A* | Green manure | *Paratrichodorus teres* | 72% reduction | Netherlands | (Hartsema *et al.*, 2005) |

##### **d) Glasshouse studies on activity of Brassica and Non-Brassica compounds**

Glasshouse studies have been conducted to validate the use of brassica and non-brassica compounds extracts to determine effective dosages and quantify nematode and damage reduction. Choice of green manure cover crops needs to consider the host status of species or cultivars to PPNs as it ensures PPN s populations decline over time during the growth of the green manure and more reduction post incorporation (Matthiessen and Kirkegaard, 2006. Glasshouse experiments provide a good opportunity to screen the host status of the different cover crops before testing performance under field conditions. The susceptibility of different cover crop species is specific to the nematode species, pathotype and even race. This has been shown in a study where *Raphanus sativus* var. Colonel and Terranova were excellent hosts for *M. hapla* but poor hosts for *M. incognita* and *M. javanica*. Variations in susceptibility of *R.sativus* var. colonel and var. Adagio were shown where Colonel was an excellent host to *M.hapla* while Adagio was a poor host to all Meloidogyne species. (Scott and Antoon, 2014).

A pot experiment comparing different dosages of *Brassica macrocarpa* leaf flour, which is known to contain high levels of sinigrin, recorded a 50% reduction in root galling index on tomato plant roots in pots treated with sinigrin when compared to the untreated control (Argento, Melilli and Branca, 2019). In a similar experiment, the viability of *Globodera pallida* encysted eggs was significantly reduced when sinigrin GSL was introduced in the soil compared to non-treated pots. The role of microbial activity in degradation of glucosinolates was also investigated in the study where sinigrin GSL was introduced in soil when Indian mustard and oilseed radish were cultivated. The degradation was monitored pre-planting, pre and post incorporation of the brassicas. Microbial activity was measured to determine its contribution in production of myrosinase responsible for degradation of sinigrin. A strong correlation between *Globodera pallida* suppression by brassicas and microbial activity was recorded which was attributed to sinigrin degradation from myrosinase produced by incorporated brassica material and myrosinase produced by soil microbes that were seen to build up post incorporation of the brassicas. Significantly higher microbial activity was recorded in the unsterilized brassica incorporated treatments compared to the sterile fallow soil. The egg viability of *Globodera pallida* encysted eggs was significantly lower in unsterilized *Raphanus sativus* treated soil compared to the sterile fallow (Ngala, Woods and Back, 2015).

No effect of biofumigation on hatching of *Globodera pallida* was recorded in the pot experiment and this was attributed to inability to obtain lethal isothiocyanate concentrations in the pot experiment (Brolsma *et al.*, 2014).

Commercially available ITCs have also been assessed in pot studies. Allyl ITCs and acryloyl ITCs were applied as pre-plant and efficacy compared with chemical fumigant metam sodium and untreated control in management of *Meloidogyne javanica* in cucumber plants. Allyl and acryloyl isothiocyanates were applied to the soil at rates of 1.0 ml and 1.1 ml respectively. Results showed that there were significantly fewer galls on cucumber roots and fewer juveniles in the soil in ITC treated and metam sodium treated pots compared to the untreated control. The two ITCs were equally effective at a lower rate of 0.5 ml per kg of soil compared to metam sodium at its recommended rate (Wu *et al.*, 2011).

### **1.3.4 Allelopathic plant species**

The exploitation of crops eliciting allelochemicals has many advantages over chemical fumigants, and this can be exploited either through crop rotations, intercropping or use as green manures. Allelopathy refers to ability of plant species to produce allelochemicals, which are secondary metabolites or their products into the environment, which have negative effects on other plants or microorganisms. These allelochemicals are released either through volatilization, exudation, leaching from plant roots or through decomposition of plant residues (Halbrendt, 1996a; Dutta et al., 2019b; Barnes & Putnam, 1987). The secondary metabolites are produced by different crops and differ in chemical structure and activity against plant parasitic nematodes (Dutta, Khan and Phani, 2019).

Sudan grass (*Sorghum sudanense* (Piper) Stapf), Sorghum-Sudan grass hybrids (*S. bicolor* (L.) Moench× *S.sudanense*), and Sudan grass hybrids (*S.sudanense* × *S. sudanense*) have been shown in several glasshouse and in field studies to be effective in supressing *Meloidogyne* spp when utilised as green manures (Mojtahedi, Santo and Ingham, 1993; Matthiessen and Kirkegaard, 2006). This effect has been attributed to the cyanoglycoside compounds contained in all parts of Sudan grass known as dhurrin. Dhurrin undergoes hydrolysis to produce hydrogen cyanide which has nematicidal effects (Viaene and Abawi, 1998). The hydrolysis product of dhurrin, hydrogen cyanide, was also attributed to the suppression of the ring nematode, *Criconemoides xenoplax,* in a field experiment where *Sorghum vulgare* was incorporated as a green manure preplant. Incorporation of *Sorghum vulgare* with and without tarp was comparable with methyl bromide fumigation in suppressing the ring nematode; nematode densities for non-fumigated-alone was 50 *C. xenoplax*/100 cm3 of soil, while nonfumigated-tarp-urea was 30 *C. xenoplax*/100 cm3 of soil, sorghum-alone was 13 *C. xenoplax*/100 cm3 of soil, sorghum- tarp-urea was 43 C. xenoplax/100 cm3 of soil, or Methyl bromide fumigation was 33 *C. xenoplax*/100 cm3 of soil (Nyczepir and Rodriguez-Kabana, 2007). Sudan grass cultivars grown as green manures showed variation in their suppression of *Meloidogyne hapla* and *Meloidogyne chitwoodi* race 1 and race 2. All Sudan grass cultivars tested were able to effectively suppress the juvenile stages of *Meloidogyne hapla* but *Meloidogyne incognita* race 1 and 2 was able to reproduce in some cultivars except for the cultivars Trudan 8 and Sordan 79 which suppressed it in both glasshouse and field experiments. The juveniles of *Meloidogyne* spp. were seen to be more sensitive than the egg masses in glasshouse studies (Mojtahedi, Santo and Ingham, 1993). In studies evaluating effect of dhurrin concentrations on juvenile mobility and hatching of *Meloidogyne incognita*, *Sorghum hybrida* cv. Super dolce showed inhibitory effects in the hatching of *M. incognita.* The dhurrin concentration able to cause the death of 50% J2 population in 24 h (LD50) was 0.58 Mm. A 50% egg hatch inhibition was recorded post exposure of egg masses to 0.38mM of dhurrin concentration, when *M. incognita* hatch was monitored 7 days after treatment, in comparison to untreated phosphate buffer. Under glasshouse conditions, *Sorghum hybrida* and *Eruca sativa* were compared in their efficacy to suppress *M. incognita* and both proved to be poor hosts (Curto *et al.*, 2012). An experiment conducted to examine dhurrin and its decomposition products, proved that indeed the hydrogen cyanide produced as a result of dhurrin hydrolysis is responsible for the suppression of *Melidogyne hapla*.

Winter rye (*Secale cereale*) has been frequently used as a cover crop in many rotation systems due to its allelopathic effects on soil borne pests and pathogens. It is also recognised for its beneficial agronomic properties, such as reducing soil erosion, increasing nutrient sequestration as well as being suppressive to weeds, and plant parasitic nematodes (Zasada *et al.*, 2005). Rye is associated with the production of allelochemicals, which are detrimental to soil borne pathogens including PPNs; it produces secondary metabolites known as benzoxazinoids, which are also found in other plants belonging to the Poaceae, Acanthaceae, Lamiaceae, Ranunculaceae and Scrophulariaceae. This compound is released when crops are macerated/incorporated in the soil. The compound 2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)- one (DIBOA) and its breakdown product benzoxazolin2(3H)-one (BOA) has each been linked with allelopathy from rye (Barnes and Putnam, 1987; Sicker *et al.*, 2000; Sicker and Schulz, 2002). The benzoxazinoids DIBOA and DIMBOA occur as glucosides in intact rye. Rye has been reported to reduce gall formation by *Meloidogyne hapla* in a rye-tomato crop rotation (Halbrendt, 1996b). Production of hydroxamic acids is another mechanism of suppression that has been associated with rye; hydroxamic acids have been shown to exhibit acute toxicity on *Meloidogyne incognita* and *Xiphinema americanum* populations. In addition to these allelopathic properties, rye has been shown to exhibit antagonistic mechanisms to plants, bacterial, insects and fungi due to production of the secondary metabolites (Zasada *et al.*, 2005). Host status studies of rye cultivars to *Meloidogyne incognita* demonstrated that nematode reproduction is mostly influenced by cultivar selection and less by concentration of the benzoxazinoids, where some cultivars of rye had significantly lower nematode reproduction than others, despite having similar concentration of the compound (Zasada et al., 2005).

Members of the genus *Medicago* are also known to produce nematicidal compounds known as saponins. *Medicago* spp. belongs to the family Fabaceae (Faboideae) and is composed of 83 species most known being *Medicago sativa*. This genus contains diverse secondary metabolites such as alkaloids, isoflavones, naphthoquinone, coumarins and saponins. Saponins are a wide group of phytochemicals containing triterpene or steroid aglycone and are particularly abundant in members of the Fabaceae. They are made up of different glycosylated triterpenic sapogenins (aglycone moieties) such as soyasapogenols A, B and E, zahnic acid, hederagenin, bayogenin and medicagenic acid, which is the dominant sapogenin consisting of 40-70% of total aglycones, although this varies in different plant tissues (D’Addabbo *et al.*, 2011). Medicagenic and zahnic acid are the two main aglycones found in *M. sativa* (50% and 15%, respectively) and *M. arborea* (30% and 15%, respectively). Medicagenic acid is abundant in the roots of *Medicago* spp., while zahnic acid is only found in negligible amounts. Bayogenin has been mostly isolated from the shoots of *Medicago arborea*. In contrast, hederagenin (35%) and bayogenin (30%) are the dominant sapogenins in both shoots and roots of *M. arabica* (Avato *et al.*, 2006)*.* In studies investigating antimicrobial activity of saponins, it was concluded that medicagenic acid and hederagenin possibly contribute to the activity detected in *M. sativa* , *M. arborea* and *M. arabica,* respectively against medically important yeasts and Gram-positive and Gram-negative bacteria (Avato *et al.*, 2006).

Structurally, saponins may have one (monodesmosidic) or more sugar chains (bi-, tridesmosidic), linear or branched, linked to the aglycone moiety (sapogenin) on an ether or ester bond. Their occurrence in different plants is correlated to the structural type, where steroidal saponins are found in monocots and triterpenoid saponins are found in dicots (D’Addabbo *et al.*, 2011). The specific mode of action of saponins in *Medicago* spp. has not been widely studied but they have been regarded as resistance factors in defence mechanism against pathogens (D’Addabbo *et al.*, 2020). In other studies, the biological effects of saponins have been attributed to the interference with the cell permeability of organisms as they have specific interactions with the cell membrane (Tava and Avato, 2006). Other studies, have shown that exposure of root knot nematode eggs to saponins at different concentrations from *Medicago sativa*, reduced the cholesterol levels in the eggs of *Meloidogyne* spp. and increased the general crop performance and growth as compared to untreated plants (Ibrahim and Srour, 2013). The biological activity of the saponins have also been related to their structural differences where monodesmosides have been shown to be more active. The aglycone and position of the sugar molecule were also said to be important determining factors in the activity and efficacy of the saponins.

Saponins have been associated with reduction of juvenile motility and egg viability of root knot nematodes *Meloidogyne incognita*. Extracts from *Quillaja saponaria ,* with rich saponin extracts has been shown to have nematicidal effects on the dagger nematode (*Xiphinema index*) and root lesion nematode *Pratylenchus thornei,* in *in-vitro* assays (Martín and Magunacelaya, 2005) . In an experiment conducted to determine activity of saponins obtained from different *Medicago* spp, no statistical difference was observed in mortality caused by the highest concentration of saponins and the nematicide oxamyl for *Meloidogyne incognita*, *Globodera rostochiensis* and *Xiphinema index.* Tests conducted using saponin extracts from different varieties of *Medicago sativa* were shown to be strongly active against *Xiphinema index* (dagger nematode) and *Meloidogyne incognita.* Mortality of *M. incognita* juveniles was above 90% after 8 h exposure to an extract from *M. Murex* or 16 h exposure to extracts from *M. hybrida*, and *M. truncatula* at a concentration of 500 µgmL-1 *. Xiphinema index* mortality was strongly affected by saponin extracts from *M. lupulina* where a mortality of 93.3 % and 100%was achieved after 8 h and4h exposure time at concentrations of 500 µg mL and 1000 µg mL-1 saponin extracts, respectively. Up to 76% mortality of *Globodera rostochiensis* was achieved after 24h exposure time to 125 µgmL-1 saponin concentration from *M. hybrida* and *M. lupulina.* Moreover, the egg hatch of *Globodera rostochiensis* was reduced to 10-21%compared to untreated control after two weeksexposure to 1000µg mL of saponins from *M. lupulina* compared to untreated control(D’Addabbo *et al.*, 2020)*.*

The nematicidal effects of saponins from *M. arabica, M. arborea* and *M. sativa,* were studied in laboratory experiments on *Xiphinema index.* Related prosapogenins, obtained by basic hydrolysis of saponins, and sapogenins produced by acid hydrolysis of saponins were also included. As a comparison, soyasaponin I and purified aglycones from *Medicago* spp.(medicagenic acid, hederagenin and bayogenin) and a commercial mixture of saponins from *Q. saponaria,* were also included in the study. All the saponins from *Medicago* spp. induced100% mortality of *X. index* at the highest concentration(500 μg ml−1)at 8 and 48 h exposure. Crude saponins from *M. sativa roots* and *M. arabica* tops and roots resulted in significantly greater mortality after 48 h than *M. sativa* and *M. arborea* tops at the same concentration. Medicagenic acid appeared slightly more active than bayogenin, causing 52% mortality at 62.5 μg ml−1 after 48 h of treatment. Prosapogenins were more nematicidal than the related saponins and sapogenins at the same dose, except for *M. sativa* tops at the maximal concentration (Argentieri *et al.*, 2008). Results obtained from a pot experiment investigating the nematicidal activity of dry foliage and root of *Medicago sativa* and *Medicago arborea* in pot mixes to suppress *Meloidogyne incognita* and *Globodera rostochiensis* showed that both amendments were able to reduce the densities of both nematode species. In the field experiments, soil amendment with pelleted *Medicago sativa* at 20 or 40 t ha−1 increased the yield of tomato and reduced soil densities and root galling caused by *Meloidogyne incognita* and densities of *Globodera rostochiensis* compared to the non-treated control(D’Addabbo et al., 2009).

Similar results were obtained from a pot experiment study investigating different organic amendments, organic fertilizers and fenamiphos on naturally infested soil with *Meloidogyne incognita*, *Criconema xenoplax* and *Paratrichodorus* spp. Densities of *Paratrichodorus* spp were reduced in amended soil as compared to unamended soils. No differences were observed in the densities of *C. xenoplax*. Densities of *Meloidogyne incognita* were suppressed in soils amended with pea straw, potato peels, green manures, lucerne pellets and soils initially held at ambient temperature and treated with fenamiphos (Walker, 2007). Marigolds (*Tagetes* spp.) are another group of plants that have been widely studied for their ability to supress nematodes by producing compounds that are potentially allelopathic to parasitic nematodes. Alpha-terthienyl has been the major compound associated with nematicidal activity. This compound contains sulphur and is concentrated in *Tagetes* spp tissues. The activity of the compound is photoactivated or is released in response to root penetration by nematodes (Hooks *et al.*, 2010). The nematicidal effect of marigolds has been attributed also to other biologically active compounds such as essential oils which are believed to be working in combination with α-terthienyl (Dutta, Khan and Phani, 2019). These mechanisms may work separately on in combination either as non-host, poor-host, allelopathy, trap crop or facilitation of other antagonistic flora and fauna (Wang et al., 2001). Marigolds have been mostly exploited against endoparasitic nematodes, such as root lesion nematodes (*Pratylenchus* spp.) as either an intercrop or in rotations schemes. This is because root peroxidases are produced in response to nematode penetration in the absence of light, which is the main activation factor. Therefore, nematodes that do not penetrate the root system i.e. ectoparasites may not be affected by the α- terthienyl biocidal compound (Bakker et al., 1979; Gommers, 1972). Marigolds also act as trap crops where they cause arrested development of juveniles e.g. juveniles of *Meloidogyne* spp (Daulton and Curtis, 1963; Ploeg and Maris, 1999).

The influence of *Tagetes patula* among other green manures such as *Crotolaria juncea*, *Chloris gayana* and *Digitaria decumbens* was investigated under glasshouse house conditions using field infested soil with *Rotylenchulus reniformis.* Results from root penetration observations showed that penetration and development of Hawaiian *Rotylenchulus reniformis* was inhibited upon planting of *Tagetes patula*, this effect was attributed to the poor host status of *Tagetes patula* which reduced the number of nematodes in the soil better than the fallow control (Caswell *et al.*, 1991).

Marigolds have the ability to supress a wide array of endoparasitic nematodes, though results can be inconsistent and there is little evidence of suppression for ectoparasitic nematodes (Hooks *et al.*, 2010). In field studies, evaluating the efficacy of summer cover crops on *Tylenchorynchus claytoni*, *Trichodorus christiei*, *Pratylenchus brachyurus*, *Helicotylenchus dihystera,* and *Xiphinema americanum*, marigolds were panted in rotation with tomato transplants, with tomatoes being grown every third year. Marigold suppressed all nematode species except for *X. americanum* which increased during the 5th year of the study (Brodie, Good and Jaworski, 1970).

The results from a glasshouse and field study investigating the efficacy of marigolds in combination with vetch on the ectoparasitic nematodes *Belanolaimus longicaudatus* (Sting nematode), *Dolichodorus heterocephalus* (awl nematode) and *Paratrichodorus allius* (stubby root nematode) showed that marigolds were excellent hosts to the nematodes as numbers increased. The nematodes densities on vetch and snap beans, were constant as no increase or decrease was recorded indicting that they were poor host to stubby root nematodes (Rhoades, 1980).

### **1.3.5 Fungal endophytes in PPN management**

Fungal endophytes colonise plant tissue to initiate a beneficial relationship that does not negatively affect the plant. They have been successfully used as biological control agents for nematode management as they protect the crop through production of bioactive compounds/secondary metabolites (antibiosis), that can kill, repel, paralyse or interfere with host finding ability, disrupt nurse cell development (Poveda et al., 2020). Some endophytes affect nematodes indirectly by competing for plant resources. Endophytic fungi are classified either as balanciaceous or non-balanciaceous depending on their ecology. Most grass endophytes fall into the category of balanciaceous endophytes whereby they are phylogenetically related e.g. the genera *Epichloe* and *Balansia* (anamorphs *Neotyphodium* and *Ephelis,* respectively) within the phylum ascomycota (Clay and Schardl, 2015). Cool-season grasses (family Poaceae) are often associated with claviceptaceous endophytic fungi in the genus *Epichloë* (F Meyer *et al.*, 2020).They are obligate symbionts and are transmitted both vertically and horizontally and grow hyphae grows intercellularly in the host plants producing structures that help uptake nutrients into their mycelia. The hyphae eventually colonise the new seed when the host reaches maturity (Christensen *et al.*, 2008).The host benefits in from the symbiotic relationship e.g. increased uptake of nutrients, increased vigour during drought, and production of metabolites that shield it against parasites and herbivores (Christensen *et al.*, 2008; Schouten, 2016). *Epichloe* spp are capable of producing bioactive alkaloids such as per-amines, lolines, ergot alkaloids and indole-diterpenes which protect the host plant from herbivory by animals, insects and nematodes. *Epichloe* spp mainly form symbiotic relationships with cool season grasses such as tall fescue (Clay and Schardl, 2015). Studies have also shown that *Epichloë* (syn. *Neotyphodium*) spp. helped in inducing resistance in perennial ryegrass where colonised plants experienced increased plant growth, reproduction and resistance to various biotic and abiotic stress factors (Esqueda et al., 2017).

However, the endophytes can produce indole-diterpene alkaloids and ergot alkaloids in pasture grasses, resulting in toxicity to livestock (Schardl et al., 2004). Associations have developed with fungal endophytes that produce little or no ergot alkaloids or the indole diterpene alkaloid lolitrem B (Timper and Bouton,2012; Young et al., 2013; Fletcher et al., 2017). *Epichloë uncinata* is a fungal endophyte which has a natural mutualistic association with meadow fescue (*Festulolium* spp) and has been studied in management of insect pests such as root aphid, pasture mealy bug, black beetle, grass grub and argentine stem weevil in New Zealand, where the loline alkaloids produced act as insect feeding deterrents (Popay and Thom, 2009)

*Festulolium* hybrids are intergeneric crosses between *Festuca pratensis* (Huds.) and *Lolium perenne* (L.) and/or *L. mulitflorum* (Lam.). *Epichloë uncinata* which colonises these hybrids, produces bioprotective loline alkaloids, which can accumulate to 2% of the host plant dry weight (Zhang et al., 2009). The loline alkaloids are water soluble and able to translocate around host tissues to areas such as the roots, where the endophyte itself is not found actively growing (Patchett et al., 2008). Importantly, loline alkaloids do not cause the animal health disorders (fescue toxicosis and ryegrass staggers) in grazing livestock associated with some of the other endophyte produced alkaloids, such as ergovaline and lolitrem B (Gooneratne *et al.*, 2012; Fletcher *et al.*, 2017). The mode of action exhibited by secondary metabolites released by some fungal endophytes against for example nematodes can be direct kill, paralysis, repulsion or impairment of the nematode host finding ability therefore death due to starvation (Clay and Schardl, 2015)

Majority of the balanciaceous endophytes are known for their antagonistic action against parasitic nematodes due to production of the bioactive compounds which are toxic. Some also induce immunity in the host plant to migratory and sedentary PPNs.

Loline, ergovaline and α-ergocryptine have nematicidal activity while ergonovaine have nematostatic activity (Schouten, 2016). However, studies with plant-parasitic nematodes and lolines indicated that the loline alkaloid N-formylloline could either attract or repel the plant-parasitic nematode *Pratylenchus scribneri*, depending on the loline concentration (Bacetty *et al.*, 2009) While endophytes can affect susceptibility of grasses to nematodes, host status may be more strongly influenced by plant cultivar than by presence or absence of endophyte, a study conducted to test the host status of *Festulolium* lines with or without the fungal endophyte. *Epichloe uncinata*, showed that the *Festulolium* lines were poor hosts with or without the fungal endophyte. *In-vitro* studies with methanolic extracts from roots and shoots of the tested *Festulolium* lines indicated that the extracts had nematicidal effects on the second stage and third stage juveniles of *Meloidogyne incognita*, where the juveniles were killed in absence or presence of the endophyte, concluding that other plant metabolites such as phenolic compounds associated with the *Festulolium* lines might be associated with the nematicidal activity. (F Meyer *et al.*, 2020)

The fungal endophyte *Neotyphodium coenophialum* associated with *Festuca arundinacea* was tested in *Pratylenchus scribinieri,* a nematode pest of tall fescue. Glasshouse host status assessments indicated that the endophyte infected fescue was a non-host (113-132 nematodes/pot) for *P.scribinieri* when compared to nil-endophyte grass.(350/pot) (Bacetty *et al.*, 2009). The study also investigated the role played by phenolic compounds in the tall fescue as well as compounds produced by the *Neotyphodium coenophialum* in *in vitro* assays. Endophyte infected grass extracts were compared to non-infected. Phenolic compounds tested showed a nematostatic effect where nematodes recovered after subsequent transfer and incubation in fresh water. The loline and ergot alkaloids were tested separately and results indicated that both were able to cause 100% reduction in nematode motility at 72-h exposure time. However the effect by lolines was seen to be nematostatic while the ergot had a nematicidal effect even at 5µg/ml concentration, when the alkaloids were combined, there was an additive effect and the effect was nematicidal indicating that they synergistically worked for effective control.(Bacetty *et al.*, 2009)

A study investigating the role of the endophyte (*Neotyphodium coenophialum*) in management of the root knot nematode *Meloidogyne marylandii*, showed that the endophyte enhanced drought tolerance of the host crop (*Festuca arundinacea*) which contributed to the protection of the root damage by the nematode A pot experiment was conducted using three endophyte infection treatments and three cultivars of perennial rye grass. Also showed the potential f endophytes in suppression of parasitic nematodes *Paratylenchus* spp Infested soil was used in this experiment and nematode population dynamics assessed. *Paratylenchus* spp numbers were significantly higher in the endophyte free treatment compared to the endophyte treatment designated as wild type endophyte (*Neotyphodium lolii*) (Eerens *et al.*, 1998)

## **1.4 Conclusion**

From the literature discussed, cover crops obtained from diverse plant families have been shown to be potential in management of different nematode species. Most of the knowledge covers endoparasitic and migratory endoparasitic nematodes e.g. root knot nematodes (*Meloidogyne* spp), cyst nematodes (*Globodera* and *Heterodera* spp) and root lesion nematodes (*Pratylenchus* spp). Limited information exists on use of cover crops in management of free living ectoparasitic nematodes, despite their huge contribution to yield loss. Currently, there is much speculation over the impact of cover cropping on free-living PPN species. Several seed suppliers claim that their cultivars will reduce nematodes, but there is also some anecdotal feedback to suggest the contrary. It is possible that the confusion is due to generalisation and a lack of knowledge about specific nematode-plant interactions. However, there is little, if any, research conducted with UK sugar beet crops to support appropriate use of cover crops for this purpose. The stubby root nematodes (*Trichodorus* and *Paratrichodorus* spp) have a wide host range. The research will focus on development of multiple nematode management strategies that can be recommended in an integrated nematode management programme to sugar beet growers. The research will explore the use of cover crop species and fungal endophytes in suppression of SRN. Cover crops may reduce nematode population densities through being poor hosts (trap crops), biofumigants (certain brassica species) or releasing toxic compounds from their roots (allelopathy) (Ntalli & Caboni, 2017). Such mechanisms of suppression require thorough investigation. Integrating endophytes with cover crop species will also be investigated. Exploration of integrated management of cover crops and endophytes will be key in development of a strategy that has multiple mode of actions against mixed populations of free-living parasitic nematodes occurring in sugar beet fields. Study objectives of the research will include:

1. Formulation of a list of candidates cover crop species, based on commercially available cover crops and host status of the cover crop the literature.
2. Conduct glasshouse experiments on hosts status of the candidate cover crop species by assessing nematode reproduction before and after planting of the cover crops
3. Conduct field experiments to assess the performance of cover crop species in suppression of stubby root by comparing population dynamics of the nematodes before, during and after cover crop maceration and incorporation.

# **2. Data and methods**

## **2.0 Introduction to data and methods**

To investigate the objectives, two experiments were carried out. A glasshouse experiment was conducted to screen the host status of sixteen cover crop species from diverse plant families. The cover crops were selected based on literature review search on their host status to *Trichodorus* and *Paratrichodorus* spp. as well as their commercial availability. For most of the cover crops, the host status was unknown, as very limited research has been undertaken on the host status of different cover crops to SRN. A field experiment was also conducted in Suffolk, Bury St. Edmunds to evaluate the potential of biofumigant brassica cover crops in suppression of SRN, when grown as a green manure. The site was selected based on previous history of occurrence of SRN. Data on initial nematode densities was collected by soil sampling prior to drilling of the biofumigants. Evidence of attraction/repulsion of the biofumigant cover crops was also of interest in the field study, and therefore soil samples were also collected 4 weeks after drilling of the cover crops. To validate the methods used in extraction of SRN, two extraction methods, namely centrifugal floatation and the Seinhorst two flask method (Bezooijen, 2006), were compared, to assess extraction efficiency (nematode yield), time per sample and ease of use. This allowed selection of an extraction method and extraction fluid for use in subsequent extractions in the experiments.

## **2.1 Experiment 1: Selection of nematode extraction method**

Two nematode extraction methods, i.e. Seinhorst two flask method (Bezooijen, 2006) and centrifugal floatation method (Harrison and Green, 1975), were compared for their efficiency in extraction of stubby root nematodes. The principal behind the Seinhorst two flask method is the difference in size, shape, and sedimentation rate between nematodes and soil particles. Soil is first passed through a 2mm sieve to remove debris and large stones. The soil is then washed into a 2L flask filled with water and placed upside down in another 2L flask containing water for a period of 10 mins. Nematodes are collected in a clean suspension and placed in sample bottles for identification and quantification. On the other hand, centrifugal floatation method, uses difference in specific gravity between the nematodes and other particles in a soil sample. The extraction liquid usually has a higher specific gravity than the nematode, hence the nematodes keep afloat and are decanted into sieves at the end of the extraction (Decraemer, Coolen and Hendrickx, 1979; Bezooijen, 2006). In the first experiment two extraction fluids, magnesium sulphate heptahydrate (MgS04.7H20) and Ludox, were compared to assess which one better maintains the integrity of the nematodes and also examine whether the nematode yields differ.100g of soil was subsampled from each composite sample. This was gently mixed to achieve a homogeneity. Nematodes were then extracted using the above-mentioned methods and replicated three times for each method. Stubby root nematodes (*Trichodorus* and *Paratrichodorus* spp) in the whole suspension were counted under a compound microscope at 10x magnification (Bezooijen, 2006; Ashmit *et al.*, 2021)

## **2.2 Experiment 2: Host status experiment on potential cover crop species**

Prior to setting up a glasshouse experiment, a cover crop candidate list was formulated based on literature screening of existing databases and articles on the host status of the different cover crops to stubby root nematodes One of the databases used was Best4Soil platform ([<https://www.best4soil.eu/>](https://www.best4soil.eu/)). Cover crops from diverse plant families were evaluated to determine their host status as shown in Table 4 to stubby root nematodes (*Trichodorus* and *Paratrichodorus* spp). Air temperature was recorded at hourly intervals using temperature data loggers. Supplementary light was provided to achieve a 16hrs long day light regime. Terracotta pots approximately 1.4 L were used. The experiment was arranged in a randomised block design of seven blocks and 17 treatments (Figure 12). Treatments were randomised using GenStat 15th Edition. Pots were directly seeded with cover crops according to the seed rate recommendations by the seed supplier as listed in Table 4. Germination was monitored 5 days after planting and continually checked after every three days to determine the percentage germination of the different cover crops. Plants were watered as required and the fallow control was hand weeded after every three days.



Figure 8: Glasshouse pot experiment on host status of different cover crop species incubated for 8 weeks after planting

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 4: List of cover crop treatments used in a glasshouse experiment to determine host status | | | | |
| Plant species | **Common name** | **Variety** | Mode of action | Seed rate kg ha-1 |
| *Trifolium Alexandrinum* | Berseem clover | Pharos | Unknown | 30 |
| *Lathyrus sativus* | Chickling vetch | N/A | Unknown | 100 |
| *Fagopyrum esculentum* | Buckwheat | Kora | Unknown | 35 |
| *Avena strigosa* | Japanese/Black oat | Bristol | Unknown | 75 |
| *Phacelia tanacetifolia* | Phacelia | Factotum | Poor host | 8 |
| *Sorghum sudanensis* | Sorghum, Sudan grass | Susu | Unknown | 235 |
| *Medicago spp* | Lucerne/ alfalfa | Etincelle | Unknown | 25 |
| *Eruca sativa* | Rocket lettuce | Tiara | Biofumigant | 8 |
| *Brassica juncea* | Brown Mustard | Brons | Biofumigant | 10 |
| *Brassica carinata* | Ethiopian mustard | Cappuchino | Biofumigant | 15 |
| *Brassica napus* | Forage rape | Greenland | Biofumigant | 10 |
| *Raphanus sativus* | Daikon radish | Longipinnatus | Biofumigant | 15 |
| *Raphanus sativus* | Oil radish | Terranova | Biofumigant | 20 |
| *Papaver somniferum* | opium poppy | Marianne | Poor host | 1.5 |
| *Lolium multiflorum* | Italian rye grass | Syntilla | Good host | 25 |
| *Festulolium spp* | Endophyte grass | Green solutions | Unknown | 25 |
| Fallow (Untreated) | Control | Control | ------- |  |

#### **Source of inoculum**

Nematode infested soil was used as a source of inoculum in this experiment. Soil sampling was conducted in nearby sites with a previous history of SRN infestation to identify a hot spot for bulk soil sampling for use in the glasshouse experiment. Three sites at Harper Adams University with a previous history of SRN infestation and two nearby farms where sugar beet was grown were selected, were also selected. A total 5 sites were sampled. The area to be sampled was divided into smaller subdivisions and sampling points evenly distributed between each subsample in order to obtain a sample of at 1-1.5 kg of soil. At each sampling point, detritus on the soil surface such as dead plant material was removed before sampling. At least 30 small cores were taken to make a composite sample. A W shape sampling pattern was used with random sampling points along the pattern. Soil was placed in labelled plastic zip-lock bag and taken to the lab for nematode extraction (Anonymous, 2009). Nematodes were extracted and quantified to enable discrete areas with higher SRN population densities identified. Approximately, 200 kg soil was collected from the area known to have high population densities of SRN; soil was carefully removed using a spade at a depth of 30cm. The soil was gently mixed and potted, before the pots were randomly allocated treatments (cover crop) and labelled accordingly.

### **2.2.1 Nematode extraction and quantification**

Prior to seeding the pots with the different cover crop species, 100 grams soil was sampled from every individual pot using a soil auger for later extraction and quantification of initial trichodorid densities per pot (Pi)**.** Eight weeks after planting, 100 grams of soil was sampled from each pot to establish the final nematode densities (Pf). Nematodes were extracted using centrifugal floatation method with MgS04.7H20. 12.5 grams soil was distributed into eight 50 ml centrifugation tubes and 20ml of MgS04.7H20 of 1.15 specific gravity added into each tube. The tubes were gently shaken to mix the soil with the extraction liquid. The sample was then centrifuged at 1150g for 5 minutes after which the supernatant was decanted into 215 µm and 53µm sieves. Nematodes were washed into 50ml sample bottles awaiting quantification. Whole nematode suspension was counted by pipetting suspension into a counting chamber, under a compound microscope at 20x Magnification. Nematode genera of the family Trichodoridae and other plant parasitic nematodes were quantified.

## **2.3 Evaluation of potential of biofumigant cover crops under Field conditions**

The objective of the experiment was to assess the potential of biofumigant cover crops in suppression of stubby root nematodes The field experiment was conducted in a site at Bury St. Edmunds, Suffolk. Plots measuring 22m by 4m were marked out. Four treatments (Table 5) were assigned across five blocks in a randomised complete block design. Blocks were spaced 6m apart to allow movement of machinery without damage to nearby plots.

|  |  |  |  |
| --- | --- | --- | --- |
| Table 5: Cover crop treatments for field experiment | | | |
| **Species name** | **Common name** | **Variety** | **Seed rate** |
| *Brassicae juncea* | Indian mustard | Brons | 10Kg/Ha |
| *Raphanus sativus* | Oil seed radish | Terranova | 20kg/Ha |
| *Raphanus sativus* | Daikon radish | Longipinnatus | 20Kg/Ha |
| Fallow | Control | Control | ------ |

### **2.3.2 Field soil sampling**

Prior to cover crop drilling, soil sampling was conducted in every plot to establish the initial nematode densities of SRN (Pi) and sampled again four weeks after drilling of the cover crop. This was done on 15m by 3m area within the plot, targeting the middle of the plot in a W sampling pattern. A total of 28 cores were sampled at a 30cm depth to obtain a composite sample of 1-1.5kg from each plot. Figure 14 shows the sampling pattern used.

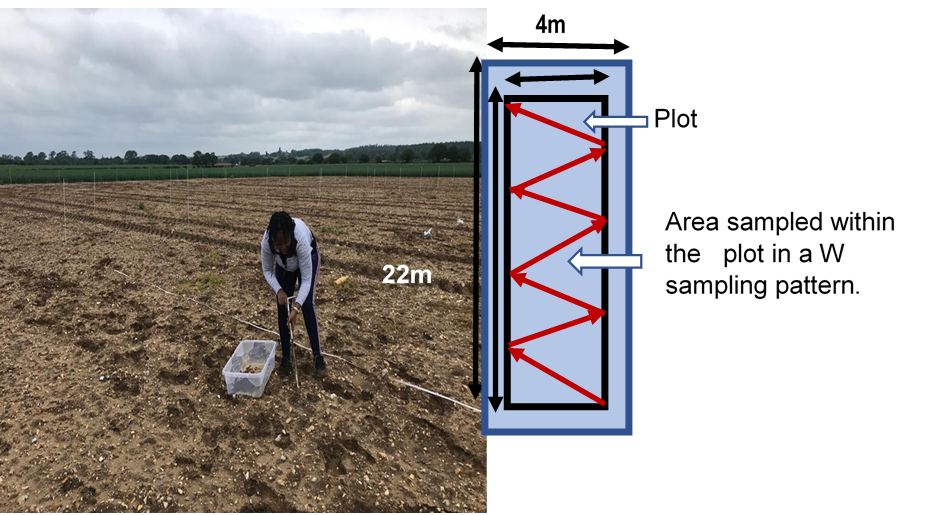
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Figure 9: Soil sampling of experimental plots at Bury St. Edmunds site, Suffolk

### **2.2.3 Nematode extraction, Identification and quantification**

Nematodes were extracted using centrifugal flotation method. The bulk sample was gently mixed and a 200ml subsample taken for extraction. The soil was divided into four 250 ml centrifuge tubes and 80ml of extraction fluid (MgS04.7H20) added into each. The tubes were gently agitated to mix the fluid and the soil and then centrifuged at 1150 g for 5 minutes extraction time. The supernatant was then decanted into 215 µm and 53µm sieves and washed into sample bottles. The suspension was concentrated into a smaller volume which was wholly quantified under a compound microscope at 20x magnification. Morphological characteristics e.g. spicule shape in males, body cuticle and vaginal characteristics of the females was used to distinguish the genus *Trichodorus* and *Paratrichodorus* spp Other prevalent PPNs in the soil sample were also quantified.

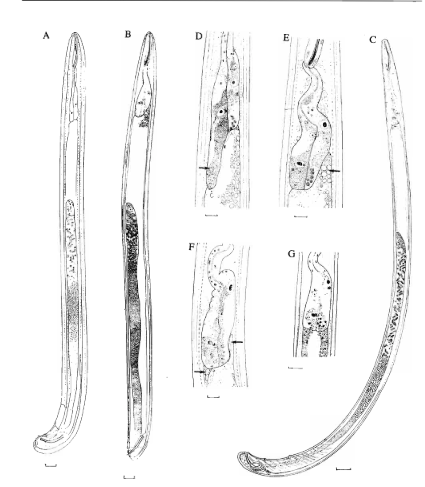
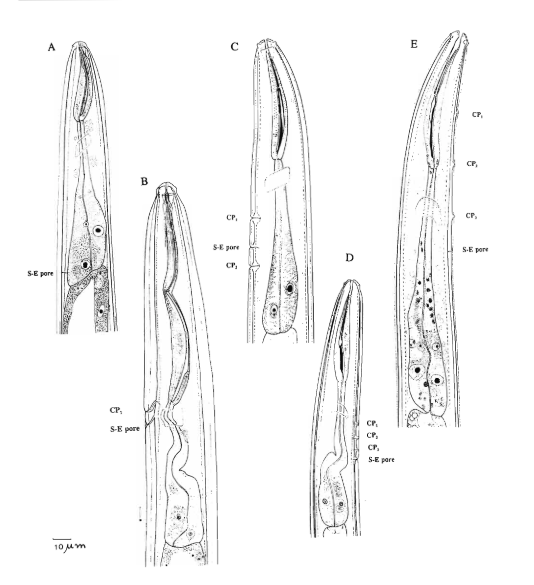


Figure 10: Polytomous key for morphological identification of nematodes in the family Trichodoridae (Decraemer and Baujard, 1998)

## **2.4 Statistical analysis**

Data was analysed using R software. Treatment effects for ANOVA analysed data were compared using Tukey’s multiple range test at 95% confidence Non-parametric data was analysed using Kruskal Wallis test and Dunn multiple comparison test used to for multiple pairwise comparison (P<0.05).

## **2.5 Results**

**Experiment 1:**

### **2.5.1 Selection of extraction method**

The first objective was to select an extraction fluid in the process of centrifugal floatation,that maintained the integrity of the nematode for ease of identification and quantification. There were no significant differences (P>0.05) between nematode densities extracted using the two fluids i.e . Both fluids equally maintained the integrity of the nematode for accurate identification and quantification (Figure 15).

Figure 11: Average densities (±SE) of Stubby toot nematodes (SRN) in100g of soil from different samples, using MgSO4.7H2O and Ludox. Vertical bars show the standard error of the mean (n = 3). \*ns = not significantly different (P> 0.05).

Results indicated that SRN densities extracted using centrifugal floatation method and the two-flask method were not significantly different (P>0.05) (Figure 16). Centrifugal floatation was selected as the extraction method for the subsequent experiments as it required less time to extract one sample (c. 5 mins) compared to two flask method (c. 10 mins/sample).

Figure 12: Average densities (±SE) of SRN in100g of soil from different samples, using Centrifugal and two flask extraction methods. Vertical bars show the standard error of the mean (n = 3). \*ns = not significantly different (p > 0.05).

### **2.5.2 Experiment 2: Glasshouse host status experiment**

#### **2.5.2.1 Source of inoculum site**

The Crabtree Leasow L9 site was identified as the potential site for mass soil sampling of the soil for use in the pot experiment as it had significantly higher SRN densities compared to the other sites as shown in Figure 17 below.

Crabtree Leasow (Maize)

Figure 13: Average Stubby root nematodes (SRN densities) in 100g of soil from five sites/ Vertical bars show the standard error of the mean (n = 3). Means with the same small letter are not significantly different (p > 0.05).

**2.5.2.2 Host status screening**

Results from the glasshouse experiment indicated no reproduction of the SRN even in the susceptible control (Italian ryegrass). There was a total decline in the final SRN numbers across all the treatments the initial SRN densities varied from one treatment to another ranging from 92 to 215 SRN per pot but the differences were not significant per treatment (P>0.05).

Figure 14: Average initial (Pi) and final (Pf) densities of stubby root nematodes (SRN) per pot (n=7) from a glasshouse experiment with 16 different cover crop species and a fallow control.

### **2.5.3 Field experiment to evaluate the potential of biofumigant cover crops in the suppression of SRN**

No significant differences in initial SRN densities were recorded, P>0.05 among all the plots prior to cover crop drilling. However, SRN population densities were significantly lower (P<0.001) in cover crop drilled plots compared to the fallow control four weeks after planting. SRN densities decreased in a similar rate in plots drilled with cover crops as no significant difference was recorded between the cover crops P>0.05. SRN densities in the fallow control increased significantly after 4 weeks as seen in Figure 19.

Figure 15: Average densities (±SE) of SRN per litre of soil, before planting (Pi) and 4 weeks after cover crop drilling. Vertical bars show the standard error of the mean (n = 5). Means with the same small letter are not significantly different according to Turkey HSD (p > 0.05).

The reproduction factor (Rf), was calculated as SRN at 4 weeks/Initial SRN (Pi). SRN reproduced significantly in the fallow control Rf=3.1 as compared to treated plots where no reproduction occurred, with RF of 0.32, 0.31 and 0.17 for Brons, Daikon and Terranova respectively (P<0.001). The Rf in the plots with different biofumigant cover crops was not significantly different P>0.05 as indicated in Figure 20.

b

a

Figure 16: Average reproduction factor (RF= density at 4weeks /Initial density (Pi) of Stubby root nematodes (SRN), Vertical bars show the standard error of the mean (n = 5). Means with the same small letter are not significantly different (p > 0.05) according to Turkey HSD.

## **2.6 Discussion**

Stubby root nematodes are economically important root ectoparasites affecting sugar beet production. Upto 50% yield losses have been recorded on sugar beet crop showing symptoms of docking disorder (Cooke and Holden, 1975). The damage caused is mainly influenced by the soil moisture conditions where severe damages have been correlated with wet months (Cooke, 1973). Management strategies used in the past in management of stubby root nematodes have mainly focused on prophylactic use of nematicides (Maughan, Cooke and Gnanasakthy, 1984), but most of these nematicides such as Vydate have been banned hence need to develop alternative management strategies (Stevens, 2015). Studies conducted in these experiments seek to develop potential cover crop species capable of suppressing SRN densities. An effective extraction method was essential in method development as the screening involves careful quantification of initial and final nematode densities. Results from the comparison experiments conducted indicated that both methods (Seinhorst two-flask and Centrifugal floatation) were effective in recovering the SRN species as no differences in densities recovered were observed. This can be explained by the fact that the two methods are based on nematode density and not motility like other extraction methods, hence matches well with the nature of SRN which are sluggish meaning they cannot be recovered by a method that requires nematode motility. In the centrifugal floatation method, the two fluids (Magnesium sulphate heptahydrate and Ludox) achieved comparable nematode densities and also equally maintained the SRN integrity for accurate quantification and identification. Magnesium sulphate heptahydrate was selected as the extraction liquid moving forward due to its affordability compared to Ludox.

Results from the glasshouse experiment on host status of the different cover crop species indicated lack of nematode reproduction, which was observed across all treatments including the susceptible control (Italian ryegrass). This result could be attributed to the sensitive nature of Trichodorids. The temperature profile recorded in the glasshouse throughout the experiment incubation, showed a spike in the temperature (36°C) at the beginning of the experiment, which might have interfered with nematode reproduction. Previous studies have shown that nematodes in the family Trichodoridae thrive well in temperatures between 21-24°C (*Trichodorus* spp) and 22-27°C (*Paratrichodorus* spp) and temperatures beyond that leads to their death or low reproduction (Rohde and Jenkins, 1957; Ayala, Allen and Noffsinger, 1969). The increase in temperature might also have affected the moisture content in the pot therefore creating an unfavourable environment for the nematodes to reproduce. Sensitivity of Trichodorid densities to moisture content in the soil have been shown in a study where high numbers were positively correlated to wet conditions in May(Jones, Larbey and Parrott, 1969; Cooke, 1973). The movement of trichodorids is also limited by moisture content. For example, a study conducted at three different moisture regimes which were categorised as either soil pores half-full of water , waterlogged or dry , demonstrated that Trichodorid movement was greatest when soil pores were half full with water and least when soil was dry or waterlogged (Bor and Kuiper, 1966; Winfield and Cooke, 1975). Trichodorids are far more susceptible to dry soil conditions than other migratory nematodes such as *Rotylenchus* spp. and *Pratylenchus* spp. (Rössner, 1971). ). The susceptibility of Trichodorids to dry soil may reflect that these nematodes are almost entirely restricted to free draining sandy soils meaning that they are likely to encounter very dry soil conditions so it is not surprising that numbers in the soil are often correlated with rainfall (Winfield and Cooke, 1975) No SRN reproduction was observed even in the susceptible control (Italian ryegrass) in this experiment, which supports the hypothesis that the conditions were not conducive for nematode reproduction. The outcome from this glasshouse experiment indicates that further controlled environment experiments need to be conducted where the soil moisture content and temperature can be constantly monitored and adjusted accordingly to create suitable conditions for SRN reproduction.

Results from the field experiment indicated the potential of brassica cover crops in management of SRN. The SRN densities decreased four weeks after cover crop drilling as compared to the fallow where the densities increased. The decrease in SRN densities in the drilled plots could be explained by the GSL and myrosinase reaction in brassicas which leads to release of ITCs, which have nematicidal effect, hence could have had negative effect on SRN. In this case, the glucosinolates might have been exuded through the young roots, which have been documented to possess high concentrations of glucosinolates during early growth. Conversely, when the plant matures, the GSLs become more concentrated in the reproductive organs i.e. flowers (Bellostas, Sørensen and Sørensen, 2004). Exudation of ITCs from actively growing roots has been reported (Elliott and Stowe, 1971) and this possibly due to superficial cell damage during active root development when the plant is young (Ngala, Woods and Back, 2015). The roots of brassicas such as oilseed rape (Choesin and Boerner, 1991) and mustard (Paul Schreiner and Koide, 1993) are also known to release GSLs into the root rhizosphere. Soil microbes in turn hydrolyse the GSL into ITCs by releasing the enzyme myrosinase (Dutta, Khan and Phani, 2019).The exuded compounds from the roots of cover crops might have had some repellent effect making the nematode migrate down the soil profile, or might have had a lethal effect of directly killing the nematode. In similar studies, the roots of *Raphanus sativus* released ITCs into the rhizosphere, which were detrimental to the encysted eggs of *Globodera pallida* (Ngala *et al.*, 2014). A higher reproduction observed in the fallow control compared to the plots drilled with the cover crops, might be an indication of nematode attraction to weeds growing in the fallow plot and therefore were able to feed and reproduce. SRN are known to be polyphagous in nature and have many weeds as hosts, which means when the host is absent, they continue feeding and reproducing on weed species present (Ayala, Allen and Noffsinger, 1969).

**Conclusion**

Preliminary results from the field experiment show the potential of biofumigants in suppression of SRN. The field experiment is still ongoing to evaluate the effect of biofumigant compounds on SRN densities upon maceration and incorporation of Brassica foliage and stems into the soil when they are 10-12 weeks old. Future work will focus on elucidating the mechanism of action employed by the brassicas in suppression of the stubby root nematodes and possible practices that can be employed to improve efficacy.

A similar field experiment is also in its early stages to evaluate brassicas and non-brassica cover crops in suppression of SRN in Docking, Norfolk.

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# **4. Appendices**

# **Appendix A: RDF Evidence report**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | **Date created** | **Evidence Type** | **Evidence title** | **Evidence link** | **Evidence text** |
| Knowledge and intellectual abilities (A) | | | | | | | | |
|  | Knowledge base (A1) | | | | | | | |
|  |  | Subject knowledge | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
|  |  |  |  | 14/09/21 | Text | Attendance of European society of nematology virtual conference |  | I attended a three days virtual European society of nematologists conference that took place from 26/05/21 to 28/05/21. This was an opportunity to get exposure to new knowledge and ongoing research in the field of nematology. It was also an opportunity to network with fellow nematologists and share ideas. |
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|  |  |  |  | 15/09/21 | File | Presentation of research topic during power point presentation training |  | Present with power\_Nyambura .pptx |
|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
|  |  | Research methods - theoretical knowledge | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  |  |  |  | 15/09/21 | File | Attended a training on power point presentation skills in scientific communication |  | Nyambura Mwangi.pdf |
|  |  |  |  | 15/09/21 | Text | Attendance of masterclass in evidence syntheses |  | I attended the training on 12th and 13th /01/2021. The training involved exposure to evidence synthesis methods that covered the key stages i.e. question setting, searching, screening, sources of bias/appraisal, coding, synthesis, etc. The training consisted of both taught elements and hands-on activities. |
|  |  | Research methods - practical application | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
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|  |  |  |  | 15/09/21 | File | Attendance of online webinar on experimental design |  | Certificate.pdf |
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|  |  | Information seeking | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
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|  |  | Information literacy and management | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  |  | Academic literacy and numeracy | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  | Cognitive abilities (A2) | | | | | | | |
|  |  | Analysing | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
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|  |  | Synthesising | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
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|  |  | Critical thinking | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  |  | Evaluating | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
|  |  |  |  | 14/09/21 | Text | Scientific writing seminar |  | Attended a virtual scientific writing webinar organized by Nemafrica on 20/08/21. The webinar covered topics on scientific writing such as Preparation and writing high-quality research papers, understanding editorial process and requirements by editors, identifying credible journals, and best practices for submitting a paper and peer review. |
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|  |  |  |  | 15/09/21 | File | Attendance of online webinar on experimental design |  | Certificate.pdf |
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|  |  | Problem solving | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  | Creativity (A3) | | | | | | | |
|  |  | Inquiring mind | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
|  |  |  |  | 14/09/21 | Text | Attendance of European society of nematology virtual conference |  | I attended a three days virtual European society of nematologists conference that took place from 26/05/21 to 28/05/21. This was an opportunity to get exposure to new knowledge and ongoing research in the field of nematology. It was also an opportunity to network with fellow nematologists and share ideas. |
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|  |  | Intellectual insight | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
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|  |  |  |  | 15/09/21 | File | Presentation of research topic during power point presentation training |  | Present with power\_Nyambura (1).pptx |
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|  |  | Argument construction | | | | | | |
|  |  |  | Phase 1 | | | | | |
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| Personal effectiveness (B) | | | | | | | | |
|  | Personal qualities (B1) | | | | | | | |
|  |  | Enthusiasm | | | | | | |
|  |  |  | Phase 1, 2 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
|  |  |  |  | 15/09/21 | File | Presentation of research topic during power point presentation training |  | Present with power\_Nyambura (1).pptx |
|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
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|  |  | Integrity | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | Text | Scientific writing seminar |  | Attended a virtual scientific writing webinar organized by Nemafrica on 20/08/21. The webinar covered topics on scientific writing such as Preparation and writing high-quality research papers, understanding editorial process and requirements by editors, identifying credible journals, and best practices for submitting a paper and peer review. |
|  |  | Self-confidence | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
|  |  |  |  | 15/09/21 | File | Attended a training on power point presentation skills in scientific communication |  | Nyambura Mwangi.pdf |
|  |  |  |  | 15/09/21 | File | Presentation of research topic during power point presentation training |  | Present with power\_Nyambura (1).pptx |
|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
|  |  | Self-reflection | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
|  |  | Responsibility | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | Text | Scientific writing seminar |  | Attended a virtual scientific writing webinar organized by Nemafrica on 20/08/21. The webinar covered topics on scientific writing such as Preparation and writing high-quality research papers, understanding editorial process and requirements by editors, identifying credible journals, and best practices for submitting a paper and peer review. |
|  | Self-management (B2) | | | | | | | |
|  |  | Preparation and prioritisation | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
|  |  | Commitment to research | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
|  | Professional and career development (B3) | | | | | | | |
|  |  | Career management | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
|  |  |  |  | 14/09/21 | Text | Attended Nematology seminar day organised by IMANEMA Ghent |  | I attended a virtual seminar day organized by IMANEMA Ghent University, Belgium via zoom meetings on 19/05/21. The theme of the seminar was career development and soft skills. The different speakers gave their experiences on pursuing a career in nematology, tips, and tricks on successful career advancement. Among the speakers were also players in the industry who explained what is expected of graduates when advancing to the job market and how to prepare for job interviews. |
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|  |  |  |  | 15/09/21 | File | Attendance of online webinar on experimental design |  | Certificate.pdf |
|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
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|  |  | Continuing professional development | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
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|  |  | Networking | | | | | | |
|  |  |  | Phase 1, 2 | | | | | |
|  |  |  |  | 22/03/21 | Link | Attendance of three days Online Biofumigation 7symposium | https://www.agroscope.ch/biofumigation7 | Attending this conference enabled me to gather new knowledge in the use of cover crops in the process of Biofumigation to suppress soil pathogens, which is relevant to my PhD project. |
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| Research governance and organisation (C) | | | | | | | | |
|  | Professional conduct (C1) | | | | | | | |
|  |  | Appropriate practice | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  | Research management (C2) | | | | | | | |
|  |  | Research strategy | | | | | | |
|  |  |  | Phase 1 | | | | | |
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| Engagement, influence and impact (D) | | | | | | | | |
|  | Working with others (D1) | | | | | | | |
|  |  | Collaboration | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
|  | Communication and dissemination (D2) | | | | | | | |
|  |  | Communication methods | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  |  |  |  | 15/09/21 | File | Power point presentation on my research topic at nematology meeting HAU |  | Nematology meeting\_2.07.2021.pptx |
|  |  | Communication media | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 14/09/21 | File | Research conference Poster presentation |  | Poster\_HAU Conference.pptx |
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|  |  | Publication | | | | | | |
|  |  |  | Phase 1 | | | | | |
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|  | Engagement and impact (D3) | | | | | | | |
|  |  | Public engagement | | | | | | |
|  |  |  | Phase 1 | | | | | |
|  |  |  |  | 15/09/21 | File | Attended a training on power point presentation skills in scientific communication |  | Nyambura Mwangi.pdf |

# **Appendix B: RDF Action plan**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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| |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | |  | | | **Phase 1** | **Phase 2** | **Phase 3** | **Phase 4** | **Phase 5** | | Knowledge and intellectual abilities (A) | | | | | | | | |  | Knowledge base (A1) | | | | | | | |  |  | Subject knowledge | Achieved | Achieved | | Achieved | | |  |  | Research methods - theoretical knowledge | Achieved | Achieved | - | - | | |  |  | Research methods - practical application | Achieved | - | - | - | | |  |  | Information seeking | In progress | - | - | | | |  |  | Information literacy and management | In progress | - | - | - | | |  |  | Languages | - | - | - | | | |  |  | Academic literacy and numeracy | In progress | - | - | | | |  | Cognitive abilities (A2) | | | | | | | |  |  | Analysing | In progress | - | | - | | |  |  | Synthesising | In progress | - | - | | | |  |  | Critical thinking | In progress | - | - | - | | |  |  | Evaluating | In progress | - | - | - | | |  |  | Problem solving | In progress | - | - | - | | |  | Creativity (A3) | | | | | | | |  |  | Inquiring mind | In progress | - | - | - | | |  |  | Intellectual insight | In progress | - | - | - | - | |  |  | Innovation | - | - | - | | - | |  |  | Argument construction | In progress | - | - | | | |  |  | Intellectual risk | - | - | | - | | | Personal effectiveness (B) | | | | | | | | |  | Personal qualities (B1) | | | | | | | |  |  | Enthusiasm | Achieved | | - | | - | |  |  | Perseverance | Achieved | | Achieved | Achieved | Achieved | |  |  | Integrity | In progress | - | - | - | - | |  |  | Self-confidence | In progress | - | - | - | - | |  |  | Self-reflection | Achieved | - | - | | | |  |  | Responsibility | In progress | - | - | - | | |  | Self-management (B2) | | | | | | | |  |  | Preparation and prioritisation | Achieved | - | - | - | | |  |  | Commitment to research | In progress | - | - | | - | |  |  | Time management | - | - | - | | | |  |  | Responsiveness to change | - | - | - | - | - | |  |  | Work-life balance | - | - | - | | | |  | Professional and career development (B3) | | | | | | | |  |  | Career management | In progress | - | - | - | - | |  |  | Continuing professional development | In progress | - | - | - | | |  |  | Responsiveness to opportunities | - | - | - | | | |  |  | Networking | In progress | | - | - | - | |  |  | Reputation and esteem | - | - | - | - | - | | Research organisation and governance (C) | | | | | | | | |  | Professional conduct (C1) | | | | | | | |  |  | Health and safety | Achieved | - | - | - | - | |  |  | Ethics, principles and sustainability | - | - | - | - | - | |  |  | Legal requirements | - | - | - | - | - | |  |  | IPR and copyright | - | - | - | | - | |  |  | Respect and confidentiality | - | - | - | - | - | |  |  | Attribution and co-authorship | - | - | - | - | - | |  |  | Appropriate practice | In progress | - | - | - | - | |  | Research management (C2) | | | | | | | |  |  | Research strategy | In progress | - | | - | | |  |  | Project planning and delivery | - | - | - | - | | |  |  | Risk management | - | - | - | - | - | |  | Finance, funding and resources (C3) | | | | | | | |  |  | Income and funding generation | - | - | - | | - | |  |  | Financial management | - | - | - | - | | |  |  | Infrastructure and resources | - | - | - | - | | | Communication, influence and impact (D) | | | | | | | | |  | Working with others (D1) | | | | | | | |  |  | Collegiality | - | - | - | - | | |  |  | Team working | - | - | - | - | | |  |  | People management | - | - | - | - | | |  |  | Supervision | - | - | - | | | |  |  | Mentoring | - | - | - | - | | |  |  | Influence and leadership | - | - | - | - | - | |  |  | Collaboration | In progress | - | - | - | | |  |  | Equality and diversity | - | - | - | - | | |  | Communication and dissemination (D2) | | | | | | | |  |  | Communication methods | In progress | - | - | - | | |  |  | Communication media | In progress | - | - | - | - | |  |  | Publication | In progress | - | - | - | - | |  | Engagement and impact (D3) | | | | | | | |  |  | Teaching | - | - | - | - | | |  |  | Public engagement | In progress | - | - | - | | |  |  | Enterprise | - | - | - | - | | |  |  | Policy | - | - | - | - | - | |  |  | Society and culture | - | - | - | - | | |  |  | Global citizenship | - | - | - | | - | |

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| **Task Name** | **Year 2021** | | | **Year 2022** | | | | | | | | | |
| **October** | **November** | **December** | **January** | **February** | **March** | **April** | **May** | **June** | **July** | **August** | **September** | **October** |
| Drilling of sugar beet (field experiment - season 1) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Nematode extraction and quantification-Initial nematode densities at sugar beet planting (Field experiment Season 1) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Nematode extraction and quantification-Final nematode densities at sugar beet harvest (Field experiment Season 1) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Drilling of cover crop in the field (Field experiment Season 2) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Cover crop Incorporation |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Nematode extraction and quantification- nematode densities Pre-incorporation (field experiment season 2) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Nematode extraction and quantification- nematode densities Post-incorporation (field experiment season 2) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *In vitro* experiments with cover crop extracted compounds |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Colloquium abstract year 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Host status experiment (Phytotron set-up) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *In vitro*-nematode culturing |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Glasshouse experiment on agronomic modifications to improve nematode suppression |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Year two report (manuscript submission) |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **Appendix C: Gannt chart for October 2021 to October 2022**