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DETERMINING THE EFFECTS OF PLANT EXTRACTS AND SALTRO
NEMATICIDE ON HATCHING, MORTALITY AND REPRODUCTION OF THE
SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*)

BY

BISHNU PRASAD DHITAL

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Plant Science

South Dakota State University

2020

THESIS ACCEPTANCE PAGE

Bishnu Prasad Dhital

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree.

Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Emmanuel Byamukama

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ABBREVIATIONS

μl = micro liter

$^{\circ}\text{C}$ = degree Celsius

ANOVA = Analysis of variance

cm = centimeter

df = degree of freedom

gm = gram

ha = hectares

in = inches

LSD = least significant difference

m = meters

mi = millions

ml = milliliter

mM = milli molar

P = probability

rpm = revolution per minute

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ABSTRACT

DETERMINING THE EFFECTS OF PLANT EXTRACTS AND SALTRO
NEMATICIDE ON HATCHING, MORTALITY AND REPRODUCTION OF THE
SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*)

BISHNU PRASAD DHITAL

2020

Soybean cyst nematode (SCN) is a microscopic worm that infects and feeds from the soybean roots, hence robbing the plant of nutrients, and entry wounds may facilitate other pathogens to infect the roots. Current SCN management practices include cultural, chemical, and biological methods. Cultural practices such as, crop rotation with non-host and different tillage practices are not efficient due to the persistent nature of SCN in the soil. Chemical nematicides, though effective, can be expensive, and some are not environmentally sound. SCN resistant soybean varieties are mainly derived from one source, PI 88788 and SCN population have already evolved to overcome this resistance. Plant extracts can be a safe and sustainable alternative for SCN management. This study tested the aqueous extracts from dry leaves and flowers of ragweed (*Ambrosia artemisiifolia*) and wormwood (*Artemisia absinthium*), and dry leaves of leafy spurge (*Euphorbia esula*) on SCN egg hatching and second stage juvenile (J2) mortality under laboratory and cysts development on soybean in greenhouse conditions. All the plant extracts at 5mg/ml inhibited the hatching by more than 90%. Among the plant extracts tested, ragweed dry flower at the rate of 94 mg/ml, caused 100% J2 mortality at 84 hours after application of extract. Plant extracts of wormwood dry flower dry leaf of ragweed,

wormwood, and leafy spurge at 94 mg/ml caused 90.5%, 89.4%, 86.6%, and 57.2% J2 mortality, respectively. In greenhouse test, soybean cyst nematode susceptible cultivar Williams 82 was grown on the soil from the SCN infested field and treated with 75 ml of extract per plant. Tap water was used as negative control and ILeVO nematicide seed treatment was used as positive control. Ragweed dry flower and dry leaf at the concentration of 100 mg/ml reduced the SCN cyst per gram of root as effectively as ILeVO nematicide seed treatment. These findings will be helpful in further developing and testing of plant based nematicides.

The efficacy of new chemical nematicide Saltro (pydiflumetofen) in SCN egg hatching and reproduction under lab and greenhouse condition was also investigated. Seed treatment with ILeVO was used as commercial check. Susceptible soybean cultivar Williams 82 was used in the experiment. Seed exudates collected from Saltro treated seed reduced the SCN egg hatching by 55.4% compared to double distilled water control, whereas radicle exudates reduced the egg hatching by 37.5%, when recorded at 15th day after application of exudates. In greenhouse condition, Saltro reduced the SCN cysts per plant by 90.5% at 30 days after planting (DAP) and by 80.3% at 60 DAP, compared to control. This study demonstrated that Saltro seed treatment reduces the SCN egg hatching and reproduction in comparison to the control. Our findings will be helpful to the researchers to do the further research under field conditions and farmers to make the decision while selecting the nematicides.

Key words: Soybean Cyst Nematode (SCN), Plant extracts, nematicides, management

CHAPTER 1

1 Literature Review

1.1 History of soybean production in the USA

Soybean [*Glycine max* (L.)] is one of the most important Leguminosae food crops grown in the world. It is believed to have originated from the northern and central regions of China (Hymowitz 1970; Lee et al. 2011). Samuel Bowen, a seaman in west India Company brought the soybean seed from China to Georgia in 1765 and then sent the seed to the American Philosophical Society in Philadelphia. He is thought to be the first man to introduce soybean (Chinese vetches) in North America (Hymowitz and Harlan 1983).

Soybean is second most crop grown in the Midwestern United States, after corn. Soybean is important source of protein for humans and byproducts of processed soybean can be used for animal feed, ink, biofuels, plastic, and lubricants (SoyStats2018). USA is world's top soybean producing country with the total production of 120.07 million metric tons which is 29% of global soybean production. Brazil, Argentina, India, and China are also major soybean producers with total contribution of 29%, 28%, 12%, 8%, and 7%, respectively (USDA FAS, 2018-2019).

In South Dakota, soybean is second most important crop after corn and ranks 8th major soybean producing state in USA, with the total production of 6.6 million metric tons in year 2017/18 (USDA NASS, 2018). The crop is grown almost in all 66 counties, but most of the production is in the eastern counties.

1.1.1 Constraints of soybean production

Biotic and abiotic factors directly affect the soybean growth and development which ultimately reduce the crop yield and seed quality. Abiotic factors such as nutrient deficiency, temperature fluctuations, and too high or too low soil moisture negatively affect the soybean crop production. Insect pests, weeds, and diseases are the major biotic factors affecting soybean production (Hartman et al. 2011). Most common diseases associated with soybean crop and yield loss are soybean cyst nematode (SCN) (*Heterodera glycines*, Ichinohe), various seedling diseases, Phytophthora root and stem rot (*Phytophthora sojae*), charcoal rot (*Macrophomina phaseolina*), sudden death syndrome (*Fusarium virguliforme*), soybean rust (*Phakopsora pachyrhizi*), brown spot (*Septoria glycines*) and white mold (*Sclerotia sclerotiorum*). Among all biological constraints, the soybean cyst nematode (SCN) is the most important biotic factor in reducing the soybean yield in United States and Ontario, Canada (Allen et al. 2017; Wrather and Koenning 2009; Wrather and Koenning 2006).

1.2 Origin and distribution of SCN

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is an obligatory, sedentary, endo-parasitic, microscopic worm (Niblack et al. 2006). SCN was first reported in Japan in 1915 (Hori 1915). SCN had been reported as early as in 1880 in northeast China (Liu et al. 1997 ; Noel 1992) but not confirmed. SCN had been reported prior to 1915 (Ishikawa 1916) but, by that time SCN was considered as the cyst nematode of sugar beet (*Heterodera schautii*). In 1921 SCN was named ‘Soybean yellow dwarf disease’ associated with sugar beet cyst nematode (Ito 1921). There are morphological similarities between

different species of *Heterodera* which led to the misidentification of SCN as other species of *Heterodera*. SCN was classified as pea cyst nematode (*Heterodera gottingiana*) in 1951 (Goffart 1951). *Heterodera glycines* was described as a new species in 1952 (Ichinohe 1952).

In the United States, SCN was first reported in New Hanover County, North Carolina in 1954 (Winstead et al. 1955). There are different views on the introduction of SCN into US. SCN might have been introduced into US during the introduction of Rhizobia of soybeans from China (Noel 1986) and introduction of flower bulbs and soil infested with *Bradyrhizobium spp.* from Japan (Hymowitz 1990; Spears 1955). From the first report, SCN is now found in 17 states in U.S and parts of Canada (Tylka and Marett 2017). In South Dakota (SD), SCN was first detected in 1995 in Union County (Smolik and Draper 1998) and has been found in 34 counties as of June 2020 (Byamukama 2020, unpublished).

1.3 Soybean cyst nematode crop hosts

Soybean is the most economically important host of SCN, but this nematode can reproduce on other cultivated as well as non-cultivated crop species. SCN infects over 140 genera of plants, mostly plant species from Leguminosae family but 22 non-Leguminosae families have been reported as hosts (Riggs and Warther 1992). Out of 1,152 Leguminosae species tested for the probable hosts for the SCN, 669 species were listed as host of SCN and among them 51 were listed as new host (Riggs and Hamblen 1962b). In the study of 66 plant species and varieties tested as probable hosts of SCN, five groups were formed, based on the penetration and development of SCN on those plants: 1) Plants not penetrated by nematodes, 2) penetrated but no development of nematodes, 3) penetrated and

development of few nematodes, 4) Penetrated, considerable nematode development but only few matured, 5) Penetrated and many nematodes matured. In group three there were Cruciferae, Leguminosae, and Solanaceae families with majority of Leguminosae plants. Both group four and five had Leguminosae plants. Plants in group five, in which many nematodes matured were lespedeza (*Lespedeza stipulacea* Maxim.), lupine (*Lupinus albus* L.), bean (*Macroptilium atropurpurea* and *M. lathyroides*), tepary bean (*Phaseolus acutifolius*), and adzuki bean (*Vigna angularis*) (Riggs 1987). In another study, chickpea (*Cicer arietinum* L.), crambe (*Crambe maritima* L.), cuphea (*Cuphea viscosissima* Jacq.), and nyjer (*Guizotia abyssinica*) were reported as host of SCN for the first time (Poromarto and Nelson 2010). SCN can reproduce on dry beans (*Phaseolus vulgaris* L.) belonging to different classes including pinto, navy, black, and kidney bean with varying degree of pathogenicity. Among the cultivars of dry bean, kidney bean is most susceptible to the SCN (Pormarto et al. 2011; Poromarto and Nelson 2009; Yan et al. 2017).

1.4 Soybean cyst nematode weed hosts

Detailed study on determination of alternative weed hosts of SCN conducted by Riggs & Hamblen (1966a) evaluated 286 weed species from 22 families and confirmed 164 weed species as host of SCN. In this study weed species in which SCN developed at least one cyst was considered a host. Winter weeds and cover crops such as common chickweed (*Stellaria media*), crimson clover (*Trifolium incarnatum*), henbit (*Lamium amplexicaule* L.), vetch (*Vicia sp.*), white clover (*Trifolium repens*) were reported as the host of soybean cyst nematode in Tennessee (Donald et al. 2007). Johnson et al. (2008) reported winter annual weeds including shepherd's purse (*Capsella bursa-pastoris* L.), common chickweed (*Stellaria media* L.), and small flowered bittercress (*Cardamine parviflora* L.)

as alternative SCN hosts. Poromarto et al. (2015) reported 26 weed species from 11 families as newly identified hosts of SCN. Common weed hosts mostly reported from the different states in United states are, henbit (*Lamium amplexicaule* L.) (Creech et al. 2005; Donald et al. 2007; Epps and Chambers 1958; Johnson et al. 2008; Poromarto et al. 2015; Venkatesh et al. 2000; Werle et al. 2015), common purslane (*Portulaca oleracea* L.) (Poromarto et al. 2015; Riggs 1992; Riggs and Hamblen 1966a), purple deadnettle (*Lamium purpureum* L.) (Creech et al. 2007b; Johnson et al. 2008; Poromarto et al. 2015; Riggs and Hamblen 1962a; Werle et al. 2015), field pennycress (*Thalpsi arvense* L.) (Johnson et al. 2008; Poromarto et al. 2015; Venkatesh et al. 2000). Weeds reported to be hosts for SCN among the tested ones include purple deadnettle, field pennycress, as good hosts and common mallow, shepherd's purse, white clover, Canada thistle, and common cocklebur as poor hosts (Basnet et al. 2020).

1.5 Lifecycle of SCN

There are three main stages in the life cycle of SCN: egg, juvenile and adult (Ichinohe 1955; Niblack et al. 2006). SCN has four juvenile stages and molts three times before developing as matured adult male or female (Ichinohe 1955). First stage juvenile develops inside the egg and first molting takes place inside the egg (Ichinohe 1955; Young 1992). The first juvenile molts to the second stage juvenile with exits the egg. Several factors like, temperature, moisture, pH, ionic solutions affect egg hatching of SCN (Tefft et al. 1982; Yen et al. 1995). Second stage juvenile (J2), the infecting stage, locates the soybean root through chemo-location and penetrates the soybean root hairs with the aid of a stylet (Niblack 2005). The juvenile moves through the epidermal cells and then modifies the cell wall of cells near vascular bundles (cellular hypertrophy, cell wall dissolution, and

clumping of nuclei from contiguous cells by secreting pathogenesis genes into these cells). After the alteration in cell walls, J2 forms the permanent feeding site (feeding plug) and induces a syncytium in the vascular tissue (Endo 1963, 1978, 1992; Gipson et al. 1971). J2 molts 3-4 days after infection (Niblack 2005). Sex differentiation of SCN juveniles takes place in third stage juvenile, which can be distinguished by observing the morphology of the genital primordium (Riggs and Warther 1992; Young 1992). Generally, ratio of male and female nematodes is 1:1, but this ratio may change due to the host resistance and other environmental factors (Colgrove and Niblack 2005). Last molting of male juvenile occurs at 8 to 9 days after infection while female juvenile molts for last time at 9 to 10 days after infection. Fourth- stage male juvenile nematode stops feeding and exits the root, while female continues feeding. The female starts producing eggs and swells with the developing eggs inside its body. The bulging female body bursts through the root epidermis but still attached to the feeding site (Niblack 2005). SCN is cross fertile species (Opperman and Bird 1998), mating of free-living male nematode and sedentary female still attached to the feeding site takes place outside the root. Adult female produces most of its eggs inside the body while a few eggs can be seen outside the body attached in the gelatinous matrix. Generally, average number of eggs per female is 200 but a female can produce more than 600 eggs (Sipes et al. 1992). Matured white female nematode take on the lemon shape. White females change into yellow while maturing and become brown when they die. The dead brown female is called a cyst. SCN completes the life cycle in 3-4 weeks but under controlled conditions SCN can complete lifecycle within 21 to 22 days. SCN can have 3-4 generations in one soybean growing season, depending on the growth season length

(Niblack 2005). SCN cyst and eggs may remain viable in the field up to 9 years without a host (Inagaki and Tsutsumi 1971; Riggs 2004).

1.6 Interaction of SCN with other pathogens

Several studies have documented both synergism and antagonism of SCN with other pathogens. Antagonistic interaction between SCN and Rhizobacteria was reported as early as in 1970 (Barker et al. 1972). SCN along with *Bradyrhizobium japonicum* enhanced the nodulation on soybean roots but the weight of the nodules was lower than the nodules from the roots free of SCN (Kennedy et al. 1999). SCN suppressed the binding of *Rhizobium japonicum* onto the soybean roots, this suppression of binding of Rhizobium is not due to reduction in surface area of SCN infected roots but as a result of interference of nematode with soybean lectin metabolism (Huang et al. 1984). SCN made the soybean plants more vulnerable to the Fusarium wilt (Ross 1965). Moreover, incidence of *Phytophthora sojae* was high in the field with high density of SCN (Kaitany et al. 2000). In a study of brown stem rust (*Phialophora gregata*, BRS) of soybean, varieties susceptible and resistant to BRS showed the increased incidence and severity of the disease while parasitized with SCN. In the plants parasitized with SCN, *Phialophora gregata* colonized the soybean stem earlier than normal colonization process. Wounds in the soybean roots caused by SCN might help the fungus to enter the roots (Tabor et al. 2003). In another study, soybean variety susceptible to both pathogens *F. virguliforme* [causal organism of sudden death syndrome (SDS)] and *Heterodera glycines* (SCN), Williams 82, showed a higher level of foliar SDS severity and root necrosis while planted in the soil infested with both pathogens than soil without SCN (Xing and Westphal 2006a; Xing and Westphal 2006b; Xing and Westphal 2013), but the mechanism behind the synergism between SCN and SDS is

unknown. In the interaction study between SCN and the soybean aphid (*Aphis glycines*), the soybean aphid significantly affected the reproduction of SCN, but the interaction effect between aphid and SCN varied with the population density of aphids feeding on soybean plant, soybean cultivar, and length of experiment (McCarville et al. 2014).

1.7 SCN Management strategies

Current SCN management practices include cultural, chemical, and biological methods. There are several cultural methods used to manage the soybean cyst nematode such as, crop rotation with non-host, use of resistant varieties, and different tillage practices, adjustment on the planting dates and spacing of soybean plants in the field (Niblack and Tylka 2008)

1.7.1 Host resistance/ resistant varieties

The search for the resistance genes to manage the SCN started as early as in 1960s, Caldwell et al. (1960) reported three recessive genes, rhg1, rhg2, and rhg3 resistant to SCN from soybean germplasm Peking. Later, one dominant gene- Rhg1 resistant to SCN was found in the same Peking (Matson and Williams 1965). In the verge of exploring the new genes resistant to SCN, a new dominant gene- Rhg5 was identified in soybean germplasm PI 88788 (Rao-Arelli 1994; Rao-Arelli et al. 1992). There are at least 118 plant introduction (PI) lines in the USDA Soybean Germplasm collection which are resistant to soybean cyst nematode (Arelli et al. 2000). PI lines used in SCN resistance breeding program are, PI 548402 (Peking), PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316 (Cloud) (Niblack et al. 2002). Among these, PI88788, Peking, and PI 437654 are the mainly used (Tylka and Mullaney 2002, 2010). Resistance in PI 88788 is mediated by rhg1-b. allele,

while resistance in Peking is governed by *rg1-a* and *Rhg4* alleles (Concibido et al. 2004; Joos et al. 2013a; Liu et al. 2017; Meksem et al. 2001; Mitchum 2016). More than 90% of the resistant commercial soybean varieties in United States are derived from PI 88788 (*rhg1-b* allele) (Concibido et al. 2004; Joos et al. 2013b; Schmitt et al. 2004; Shannon et al. 2004; Tylka and Mullaney 2015). In PI line 567516C, one new QTL has been discovered with broad-based resistance to SCN (Vuong et al. 2010)

1.7.2 Crop Rotation

Crop rotation is one of the mostly adopted management strategy for the SCN. SCN has a broad host range including cultivated crops and weeds, therefore in order to decrease the SCN population or inoculum in the soybean field, soybean can be rotated with non-host crops (Ross 1960) along with the control of possible weed host of SCN (Niblack and Tylka 2008; Thomas et al. 2005). In the Midwestern and North Central regions of USA, soybean-corn cropping system is common. Rotating soybean with corn reduces the SCN population in the field, while non-host Leguminosae crops not only reduces the SCN population but also increases the soybean production. But only a year rotation away from soybean may not be effective to manage the SCN (Chen et al. 2001; Miller et al. 2006; Noel and Edwards 1996; Pérez-Hernández and Giesler 2014). Rotating SCN resistant and susceptible variety affects the SCN population density and soybean yield (Francl and Wrather 1987). Different cropping pattern of soybean with SCN resistant and susceptible variety along with non-host for the six years, affected the SCN population in the field and yield of soybean (Young et al. 1986). A study conducted in Minnesota for 10 years concluded that the SCN populations was increased and yield was reduced each year in soybean monoculture compared to field with soybean-corn rotation every 5 years (Grabau

and Chen 2016). SCN density in the field with continuous susceptible variety was found to increase 2.5 times than in the field with continuous resistant variety. Rotation of soybean with corn for 3 years reduced the SCN population than the rotation for only one year and soybean yield was also high in the 3 years rotation than in one year (Sasser and Uzzell Jr 1991). Rotation of SCN susceptible soybean variety with corn greatly reduced the SCN population in comparison to the tillage and biocides (Neher et al. 2019). Chen et al. (2008) reported that sun hemp and red clover were effective to use in crop rotation with soybean as they reduced the SCN population by stimulating the egg hatching of SCN without the host. SCN is very persistent pathogen in the soil and crop rotation alone will not be effective as continuous cultivation of SCN non-host crop such as corn in the SCN infested field for five years didn't eliminate the future problem of SCN (Porter et al. 2001).

1.7.3 Chemical control

In the past years, fumigant nematicides were used most extensively than the non-fumigants with both non-systemic and systemic properties (Andersen and Sarma 2002; Duniway 2002; Kabana 1992; Rich et al. 2004; Taylor 2003). None of the nematicide completely kill the SCN in the soil but still there are some nematicides labeled for SCN, which include Telone (1,3-dichloropropene:1, 3-D), aldicarb (Temik), oxymal (Vydate), Methyl isothiocyanate (Meta sodium) (Davis and Tylka 2000; Duniway 2002; Kabana 1992). Application of aldicarb, a non-fumigant granular carbamate (Bayer CropScience and Telone C-35), a fumigant (Dow Agrosiences) were found to be effective against SCN along with soybean yield benefit in the Midwest region of USA but the efficacy of both nematicides are not consistent across the states (De Bruin and Pedersen 2008; Niblack et al. 1992; Noel 1987; Rotundo et al. 2010; Smith et al. 1991). Nematicide seed treatment

is becoming a new management strategy for SCN as this is safer than soil application of nematicides and can be combined with other fungicides and insecticides at the same time (Munkvold et al. 2014). Soybean seed treated with Avicta (Abamectin) was evaluated in Iowa for three year from 2013 to 2015 both in greenhouse and field study (small plots). This study showed the inconsistent effect on the population of SCN across the sites and soybean varieties used in this experiment (Clifton et al. 2018). In another study with ILeVO (active ingredient: fluopyram), exudates from the soybean seed treated with ILeVO reduced the egg hatching, motility of second stage juvenile (J2) and root penetration by J2, but the effect was not consistent (Beeman and Tylka 2018). In this study, seed treatment reduced the reproduction of SCN in the soybean plants up to 97% in comparison to the nontreated control. Zaworski (2014) studied the effect of ILeVO seed treatment on reproduction of SCN in greenhouse condition but did not find any significant effect. BIoST 100 soybean (active ingredient: ??) and Saltro (active ingredient: pydiflumetofen) are the new soybean seed treatment nematicides, but there is no independent published information about the efficacy of these products. Sustainable SCN management strategy without or in complement to chemical nematicide is urgently needed (Barker and Koenning 1998). Chemical nematicides are not ecofriendly measures of SCN management. Negative effect of chemical nematicides on human health and environment and high cost of the nematicides led to the widespread deregistration of several nematicides effective against plant parasitic nematodes, which reduced the application of nematicides in the field (Chitwood 2002; Gowen 1992; Kabana 1992; Matthiessen and Kirkegaard 2006; Oka 2010; Rich et al. 2004).

1.7.4 Biological control

1.7.4.1 Bacteria as a biocontrol agent for SCN

Researches exploring bacterial communities as nematodes antagonists began as early as 1980s (Jatala 1986; Mankau 1980). But the research then was more focused on root knot nematode (RKN; *Meloidogyne spp.*). Most of the bacteria tested against the RKN has been *Bacillus spp.* however, *Pasteuria penetrans* has been used extensively (Al-Shalaby and Sedik 2008; Belarmino 1998; Bird et al. 2003; Davies and Spiegel 2011; Kariuki et al. 2006; Mateille et al. 2002; Preston et al. 2003). The use of bacteria as the biological control agent in soybean cyst nematode is now an emerging field of nematode control research. The research at present is mostly focused on identifying the strains of bacteria that infect the SCN and, determining the efficacy against SCN control in the greenhouse and/or field conditions (Abd-Elgawad and Askary 2018). Xiang et al (2017a) conducted research in Alabama and tested 663 plant growth promoting rhizobia strains (PGPR) under invitro, greenhouse, microplot and field conditions. Out of 663, 608 were *Bacillus species*, and others belong to different genera. Their study concluded that *Bacillus velezensis* strain Bve2 consistently reduced SCN (*Heterodera glycines*) population under greenhouse, microplot and field conditions. This study also recommended that, combination of two strains can effectively contribute to the control of SCN, as shown by their data on SCN reduction from mixture of *B. altitudinus* (Bal13) and *Bacillus velezensis* (Bve2) strains. Combination of three rhizobacterial strains; *Bacillus simple*, *B. megaterium* and *Sinarhizobium freddii* in the ratio of 3: 1:1 respectively had shown that the reduction in the cyst number per root was 55.5% and 60.7 %; cyst per 100m³ by 14.7% and 16.5% and juveniles inside the soybean roots by 53.5% and 85.9%, respectively, at two locations of

the study (Zhou et al. 2017). In long run, Genera *Pseudomonas*, *Purpureocillium* and *Pochonia* were found abundantly in the soil, which are identified earlier to suppress SCN (Hamid et al. 2017). Hussain et al (2018) analyzed the bacterial communities in plant-associated microhabitats and SCN cysts in suppressive soil. They have reported the consistent abundance of bacteria belonging to the Genus *Pasteuria*, *Pseudomonas*, *Rhizobium*, and other taxa in suppressive soil. A novel Genus *Klebsiella pneumoniae* has been identified in 2018, which is reported to decrease the proportion of SCN, especially adult females in both greenhouse and field conditions (Liu et al. 2018). A split plot experiment on the same study determined that the effect of *Klebsiella pneumoniae* to SCN reduction is systemic, consistently blocking penetration of SCN into the root. CruiserMax Advanced, Clariva Complete Beans containing Clariva pn (*Pasteuria nishizawae*) and Poncho/ VOTiVO containing *Bacillus firmus* I-1582, etc. are some common commercial biological formulations targeted to SCN (Bayer Crop Science, 2018). But the efficacy of these products in the field are not consistent (Musil 2016). Katsande (2019) studied the effect of Aveo EZ nematicide (*Bacillus amyloliquefaciens*), *Bacillus firmus*, and Fluopyram seed treatment on the management of SCN in dry bean (*Phaseolus vulgaris* L.). This study also reported the inconsistent effect of *Bacillus firmus* on the SCN.

1.7.4.2 Fungi as a biocontrol agent for SCN management

Several fungi species have been studied for their nematocidal properties against plant parasitic nematodes including both root knot nematode (RKN) and SCN (Hu et al. 2018; Lopez-Llorca et al. 2002; Zhang et al. 2008). Bernard et al (1997) studied and isolated the fungi colonizing cyst of SCN. Among the 47 species of fungi infecting SCN cyst, *Fusarium solani* was most frequent. This study also reported that, the percentage of parasitism of

female was low in July and August but triple in September. Penetration and colonization of SCN female, cyst and gelatinous matrices by *Verticillium lecanii* study indicated the fungus started to colonize the gelatinous matrices within 16 hours after inoculation and proliferated within a week and female and cysts 3 days after inoculation (Meyer and Wergin 1998). In a study of SCN egg and J2 parasitism by the nematophagous fungus, *Hirsutella rhossiliensis*, the nematode population was reduced by 79% when J2s were used as SCN inoculum and reduced by 34% when eggs were used as inoculum. This study concluded that, parasitism by fungus is affected by the stage (egg and juvenile) of the soybean cyst nematode (Zhang et al. 2008). Nematophagous fungi are classified into four broad groups based on their mechanism of affecting nematodes (Liu et al. 2009). The four groups are (1) nematode trapping fungi: sticky hyphae and hyphal nets trap the juvenile nematodes (*Arthrobotrys oligospora*) (Swe et al. 2011), (2) Endoparasites: (Liu and Chen 2000), (3) Egg parasitic fungi, and (4) Toxin producing fungi (Liu et al. 2009).

There are several reports of attempt of mass production and commercialization of nematophagous fungi (Bíró-Stingli and Tóth 2011; Jaffee and Muldoon 1995; Lackey et al. 1993; Tranier et al. 2014; Warrior 1999), but there is little progress as the performance of the product in the field is inconsistent due to chemical, physical and abiotic factors in the soil. Growth and multiplication of fungi is very slow in the soil and decrease in virulence of fungi against nematode are the main limiting factors of commercialization of nematophagous fungi (Mo et al. 2005; Siddiqui and Mahmood 1996; Swe et al. 2011).

1.7.5 Plant extracts

Use of plant extracts can be another option for SCN management. This strategy has been widely used in the management of plant parasitic nematodes (PPN), mainly root knot nematode (RKN) of vegetables. Akhtar and Mahmood (1994) have reviewed over 100 plant species showing antagonistic effect to the specific PPN and most of the PPN reported are RKN. This review has listed different plant species; *Tagetes spp.*, *Ricinus communis*, *Datura metel*, *Leucaena leucocephala*, *Canabis sativa*, *Boehmeria nivea* *Brassica spp.*, *Azadirachta indica*, and *Argemone Mexicana*, which have been tested against PPN of various crops and a few species belonging to genus *Heterodera* but not against *Heterodera glycines*. (Chitwood 2002) has also reviewed the different plant species with various chemical compounds like alkaloids, lipids, terpenoids, steroids, isothiocyanates, etc., which are antagonistic to PPNs.

Organic amendments or organic matter with low carbon nitrogen ratio, enriched with volatile fatty acids (VAF) and ammonium nitrogen (NH_4^+) were also found effective to manage the plant parasitic nematodes (Akhtar and Mahmood 1996; Rodriguez-Kabana 1986; Xiao et al. 2007). In a study of various organic soil amendment practices for management of SCN, along with plant extracts namely marigold plant, penny cress seed powder, canola meal, *Cuphea* seed oil and spring camelia concluded that plant extract of marigold plant, pennycress seed powder and canola meal were most effective which reduced the SCN population density by 46.6%, 46.7%, and 73.2% respectively (Grabau 2013). But these plants also had the negative effect on plant height of soybean. However, a considerable finding in this research is that the effectiveness of this treatment persists only for two generations and the SCN population was again increased after two years.

Incorporation of residue of annual rye grass reduced the SCN number in the soil and soybean root in green house conditions (Riga et al. 2001). Root exudates of annual rye grass (*Lolium multiflorum*) increased the egg hatching of SCN in the absence of host, reduced the neutral lipid reserve in the juveniles, and decreased the infectivity of juveniles. Similar mechanism of bean sprout residue on the egg hatching of SCN was reported by Toyota et al., (2013). In this study, bean sprout when applied to the soil containing SCN, stimulated the SCN egg hatching and increased the number of J2s in the soil. But, in the absence of host, J2 starved to death and decreased the SCN density in the soil by 70%. Plant extracts from stem and root of *Mucuna aterrima* (annual bean) at the concentration of 5 g/ml caused 0 to 74.4% J2 mortality of SCN in in vitro test. Nematicidal property of this plant is mainly due to the presence of nitric oxide of sodium and potash, mixture of fatty acids, and mixture of triacylglycerols (Barbosa et al. 1999). Different phytochemicals isolated from both root and shoot of the *Mucuna cinerea* (Velvet bean) were used in J2 mortality test of RKN and SCN. Among the several phytochemicals, prunetin at the concentration of 50 µg/ml caused 70% J2 mortality of RKN but had no significant J2 mortality of SCN (Demuner et al. 2003). Aqueous, methanolic, and hexane extract obtained from neem was evaluated in invitro and green house conditions against SCN (Silva et al. 2008). Aqueous extract at the concentration of 4.16 mg/L and methanolic extract at the concentration of 1,000 mg/L caused more than 98% J2 mortality, showing the higher efficiency of aqueous extract than methanolic extract. Aqueous neem extract at the rate of 42.6 mg/L and methanolic neem extract at the concentration of 1000 mg/L reduced the number of females by 84% in the green house test and reduced number of eggs about 90%. Following the reverse phase high performance liquid chromatography test, seven

tetranortriterpenoids (azadirachtin H, azadirachtin A, azadirachtin B, desacetylnimbin, desacetylsalannin, nimbin and salannin) were identified from neem extract as the chemicals having nematicidal property against SCN. Auwal et al (2013) investigated the efficacy of aqueous extract of acacia (*Acacia nilotica*), neem (*Azadirachta indica*), Chinese cabbage (*Brassica chinensis*), and sea bamboo (*Ecklonia maxima*) against SCN in invitro and green house conditions. Among the four plants tested *Azadirachta indica* was found most effective. Aqueous extract from dry plant parts of *Azadirachta indica* at concentration of 120 micro gram per ml of water caused 100% juvenile mortality, inhibited hatching of egg by 75%, also reduced the number of females and cysts 83.1% and 80.1% respectively. *Brassica chinensis* was second most effective after *A. indica* and nematicidal property is mainly due to the chemicals; nitriles, thiocyanates, and isothiocyanates. Field experiment for two years with same four plant species neem, Chinese cabbage, acacia, and sea bamboo reduced the SCN population by 48.3%, 41.4%, 34.5%, and 28.3%, respectively and increased the number of pods in the soybean plants (Auwal et al. 2014).

Wormwood is a perennial herb commonly found in wild in many countries and reported to have insecticidal (Kordali et al. 2006) and anthelmintic property (Tariq et al. 2009). The effectiveness of wormwood as nematicide has been tested on Root knot nematode. Essential oils from this plant was found to be effective to inhibit egg hatching and killing second stage juveniles (J2s) of *Meloidogyne javanica* (Amora et al. 2017), whereas alcoholic and aqueous extracts of wormwood leaves killed J2s of *Meloidogyne incognita* (Liu et al. 2019). However, no reports exist on the effect of wormwood extracts on SCN. Studies have shown that ragweed (*Ambrosia trifida*) abundance in field can reduce the population of various plant parasitic nematodes (Wang et al. 1998), like Thorne's lance

nematode (*Rotylenchus robustus*) and Northern root lesion nematode (*Pratylenchus penetrans*) (Gommers 1973). Leafy spurge (*Euphorbia esula*) is a serious invasive weed in the northern Great Plains of North America. Ingenol and several diterpene derivatives from this plant showed irritant activity (Hecker 1978; Seip and Hecker 1982). Ingenol and ingenol esters appear to induce toxicity because of their ability to mimic the function of diacylglycerol and activate protein kinase C (Hasler et al. 1992; Winkler et al. 1993). Aversive activities of *E. esula* has been established in various studies (Halaweish et al. 2003; Halaweish et al. 2002; Kronberg et al. 2006). It is possible that some of these compounds may have nematicidal properties against SCN. Ethanolic extract from the leafy spurge caused 39.9% juvenile mortality of Root knot nematode (*Meloidogyne incognita*) Liu et al. (2014). However, very limited information is available on the efficacy of these plant extracts on SCN.

1.8 Justification of the study

Various management strategies have been used so far for managing soybean cyst nematode. However, these management strategies are not efficient to control SCN population in the field. Crop rotation is not effective, few years of rotation are not enough to decrease the SCN field population due to the persistence of SCN in the soil. Chemical nematicides, though effective, cannot eliminate the pathogen from the field completely (Davis and Tylka 2000; Duniway 2002). Resistant varieties have been developed, but the resistance gene incorporated in most of them is from the same source, PI 88788 (Tylka and Mullaney 2015), and the SCN populations have already evolved to overcome this resistance (Cary and Diers 2007).

Therefore, there is a need for an efficient SCN management strategy, which is cheap and easily applicable at the same time. Management strategy using the plant extract can be a possible option. Use of plant extract as a management option has been widely explored on the root knot nematode but very limited work is done in SCN in comparison. In this study, plant extracts from three commonly available weed species; wormwood, ragweed and leafy spurge will be tested against SCN for their nematicidal property.

The findings of this research will be helpful to researchers and farmers, who are seeking efficient solution for management of SCN.

1.9 Objectives

The broad objective of this project is to explore plant extract as alternative management strategy against SCN. The specific objectives are as follows:

- I. To determine the nematicidal properties of plant extracts in vitro (SCN egg hatch and second stage juvenile mortality) test
- II. To determine the effect of plant extracts on the development of soybean cyst nematode on soybean plants under greenhouse conditions
- III. To determine the efficacy of Salstro nematicide in lab and greenhouse conditions

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CHAPTER 2

2 Determining the effect of plant extracts on hatching, mortality and reproduction of the soybean cyst nematode (*Heterodera glycines*)

Abstract

Soybean cyst nematode (SCN) is one of the most economically important pathogen of soybean crop around the world. Among different management strategy, use of plant extracts can be a safe and sustainable alternative for SCN management. In this study, we tested the aqueous extracts from dry leaves and flowers of ragweed (*Ambrosia artemisiifolia*) and wormwood (*Artemisia absinthium*), and dry leaves of leafy spurge (*Euphorbia esula*) on SCN egg hatching, second stage juvenile (J2) mortality under laboratory conditions and on cysts development under greenhouse conditions. In in-vitro egg hatching test, 50 eggs were incubated in 250 μ l of plant extract with varying concentrations and in distilled water as the check. In J2 mortality test, eggs were replaced with 30 J2s. In first run, plant extracts of ragweed dry flower, leafy spurge dry leaf, and wormwood dry flower at 5mg/ml concentration reduced the SCN egg hatching by 83.3%, 79.3%, and 73.6%, respectively. While in the second, all the plant extracts at 5mg/ml inhibited the hatching by more than 90%. Among the plant extracts tested, ragweed dry flower at the rate of 94 mg/ml, caused 93.3% J2 mortality at 84 hours after application of extract in the first run, while there was 100% mortality in the second run. In the second run plant extracts of Wormwood dry flower and dry leaf extract of ragweed, wormwood, and leafy spurge at 94 mg/ml caused 90.5%, 89.4%, 86.6%, and 57.2% J2 mortality, respectively. Whereas percentage J2 mortality was relatively low (36.7-93.3%) in first run

for all the plant extracts tested. In greenhouse test, soybean cyst nematode susceptible cultivar Williams 82 was grown on the soil from the SCN infested field and treated with 75 ml of extract per plant at the rate of 100 mg/ml and 50 mg/ml. Tap water was used as negative control and ILeVO nematicide seed treatment was used as positive control. Floral extracts of Ragweed, and wormwood, and leaf extracts of ragweed, wormwood, and leafy spurge at the concentration of 100 mg/ml reduced the SCN cysts per plant by 97.4%, 90.6%, 94.1%, 83.8%, and 71.7%, respectively in the first run relative to the untreated check (tap water). Whereas reduction in SCN cyst per plant was relatively low (84-53.7%) in second run for all the plant extracts tested. Ragweed dry flower and dry leaf at the concentration of 100 mg/ml reduced the SCN cyst per gram of root as effectively as ILeVO nematicide seed treatment in both runs. Among the plant materials tested, ragweed dry flower was showing better nematicidal property than others. These findings will be helpful in further developing plant based nematicide.

Key words: Soybean Cyst Nematode (SCN), Plant extract, nematicidal

2.1 Introduction

Soybean [*Glycine max* (L.) is one of the most important Leguminosae food crops grown in the world. It is important source of protein for humans, and byproducts from processed soybean are used for animal feed, ink, biofuels, plastic, and lubricants (SoyStats2018). Soybean is second most grown crop, after corn in United States (USDA FAS, 2018-2019). South Dakota ranks 8th major soybean producing states in US, with the total production of 6.6 million metric tons in year 2017/18 (USDA NASS, 2018). The crop is grown almost in all 66 counties, but most of the production is in the eastern counties.

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is an obligatory, sedentary, endo-parasitic, microscopic worm (Ichinohe 1952), that infects and feeds from the soybean roots, hence robbing the plant of nutrients (Riggs 2004). The entry wounds created by SCN while entering soybean roots may facilitate other pathogens to infect the roots (Xing and Westphal 2013). Among all abiotic and biotic factors affecting soybean production, soybean cyst nematode is the most important biotic factor reducing the soybean yield in United States (Allen et al. 2017; Hartman et al. 2011; Wrather and Koenning 2009; Wrather and Koenning 2006).

Current SCN management practices include cultural, chemical, and biological methods. There are several cultural methods to manage the soybean cyst nematode such as, crop rotation with non-host, use of resistant varieties, and different tillage practices, adjustment on the planting dates and spacing of soybean plants in the field (Niblack and Tylka 2008). However, these management strategies are not efficient to control SCN population in the field. Crop rotation is not very effective since few years of rotation are not enough to

decrease the SCN field population. SCN is very persistent pathogen in the soil and crop rotation alone will not be effective as continuous cultivation of a non SCN host crops such as corn in the SCN infested field for five years did not eliminate the future problem of SCN (Porter et al. 2001). Chemical nematicides, though effective, can be expensive and some are toxic to the environment. (Davis and Tylka 2000; Duniway 2002). Resistant varieties have been developed, but the resistance gene incorporated in most of these varieties is mainly from one source, PI 88788 (Tylka and Mullaney 2015), and the SCN populations have already evolved to overcome this resistance (Cary and Diers 2007).

Therefore, there is a need for an efficient SCN management strategy that is cheap and easily applicable. Use of the plant extracts in the management of SCN can be a possible option. Application of plant extracts as a management option has been widely explored on the root knot nematode (Amora et al. 2017; Julio et al. 2017; Li et al. 2018) but very limited work has been done in SCN. In earlier studies using plant materials, fresh chopped marigold plants, pennycress seed powder and canola meal were found effective in reducing the SCN population density by 46.6%, 46.7%, and 73.2%, respectively (Grabau 2013). But these plant treatments stunted soybean growth and the effectiveness of treatments persisted only for two crop cycles. In vitro test using plant extract of stem and root of annual bean (*Mucuna aterrima*) caused up to 74.4% J2 mortality of SCN. Nematicidal property of this plant is mainly due to the presence of nitric oxide of sodium and potash, mixture of fatty acids, and mixture of triacylglycerols (Barbosa et al. 1999). Similarly, extracts of neem plant (*Azadirachta indica*) at various concentrations have been reported to cause up to 98% J2 mortality, 84% reduction in number of SCN females, and 90% reduction of eggs in greenhouse experiments (Silva et al. 2008). In addition, aqueous extracts of neem

(*Azadirachta indica*), Chinese cabbage (*Brassica chinensis*), acacia (*Acacia nilotica*), and seaweed (*Ecklonia maxima*) reduced the SCN population by 48.3%, 41.4%, 34.5%, and 28.3%, respectively and increased the number of pods in the soybean plants by 58.7%, 33.7%, 28%, and 37.9%, respectively in field experiment conducted for two years (Auwal et al. 2014).

Wormwood is a perennial herb commonly found in wild in many countries and reported to have insecticidal (Kordali et al. 2006) and anthelmintic property (Tariq et al. 2009). The effectiveness of wormwood as nematicide has been tested on Root knot nematode. Essential oils from this plant was found to be effective to inhibit egg hatching and killing second stage juveniles (J2s) of *Meloidogyne javanica* (Amora et al. 2017), whereas alcoholic and aqueous extracts of wormwood leaves killed J2s of *Meloidogyne incognita* (Liu et al. 2019). However, no reports exist on the effect of wormwood extracts on SCN. Studies have shown that ragweed (*Ambrosia trifida*) abundance in field can reduce the population of various plant parasitic nematodes (Wang et al. 1998), like Thorne's lance nematode (*Rotylenchus robustus*) and Northern root lesion nematode (*Pratylenchus penetrans*) (Gommers 1973). Leafy spurge (*Euphorbia esula*) is a serious invasive weed in the northern Great Plains of North America. Ingenol and several diterpene derivatives from this plant showed irritant activity (Hecker 1978; Seip and Hecker 1982). Ingenol and ingenol esters appear to induce toxicity because of their ability to mimic the function of diacylglycerol and activate protein kinase C (Hasler et al. 1992; Winkler et al. 1993). Aversive properties of *E. esula* has been established in various studies (Halaweish et al. 2003; Halaweish et al. 2002; Kronberg et al. 2006). It is possible that some of these compounds may have nematicidal properties against SCN. Ethanolic extract from the leafy

spurge caused 39.9% juvenile mortality of Root knot nematode (*Meloidogyne incognita*) Liu et al. (2014). However, very limited information is available on the efficacy of these plant extracts on SCN.

In our study, we used aqueous plant extracts from three commonly available weed species in South Dakota; wormwood (*Artemisia absinthium*), ragweed (*Ambrosia artimisiifolia*), and leafy spurge (*Euphorbia esula*) to test against SCN for their nematicidal properties. The findings of our study will be helpful to develop plant based nematicides.

Objectives of our study were to determine nematicidal properties of three plant extracts in vitro SCN egg hatch and second stage juvenile mortality test and to determine the effect of plant extracts on the development of soybean cyst nematode on soybean plants under greenhouse conditions.

2.2 Material and methods

2.2.1 Plant materials

Wormwood (*Artemisia absinthium*), ragweed (*Ambrosia artemisiifolia*), and leafy spurge (*Euphorbia esula*) plants (Figure 2.1) were collected from the different places of Brookings in Summer. Wormwood and ragweed were collected mostly from the Dakota Nature Park, Brookings, SD. Leafy spurge was collected from the grassland around the SDSU campus, Brookings. Leaf and flower parts were collected for wormwood and rag weed plants, while only leaves were collected for the leafy spurge. Plant materials were separated into leaf and flowers immediately after the collection from the field. Leaves and flowers were washed

in tap water and left to dry for 12 hours in the laboratory. These were further dried in the greenhouse for 2-3 days to prepare dry plant extract.

2.2.2 Preparation of plant extract:

Plant materials dried in the greenhouse were crushed and weighed. A 1:10 weight by volume was used to prepare the extracts. One gm of dry crushed plant materials was soaked in 10 ml of double distilled water (ddH₂O) for 24 hours. The soaked plant materials were blended in electric blender (Waring Commercial, Connecticut, USA) for 2 minutes. Blended plant material was first filtered with cheese cloth and then centrifuged (Beckman Coulter; Avanti JXN-30) @ 10,000 g (for 20 minutes). Centrifuged extracts were filtered using syringe filter (Millex® Syringe Filter Units, Sterile, 33mm) and the filtrate was kept in the freezer at -20° C until used in the experiments. Syringe filtered extract was only used in the in-vitro tests, while centrifuged extract was used in the greenhouse experiments.

2.2.3 Soybean cyst nematode inoculum:

Soybean variety, Williams 82 was used to multiply and maintain the soybean cyst nematode in the greenhouse inoculum required for the lab and greenhouse experiments. SCN cysts were extracted from 4-8 weeks old plants. Each plant was uprooted, and roots were sprayed with a strong stream of water to dislodge the SCN cysts. SCN cysts were collected in a 250- μ m sieve nested under a 710- μ m sieve. Females and cysts were surface sterilized with 0.5% NaOCl for 3 minutes and rinsed with double distilled water for three times (Hu et al. 2018). The cysts were then crushed using motorized rubber stopper to release eggs, and eggs were collected in 25 μ m sieve nested under 75 μ m sieve (Faghihi

and Ferris 2000). The obtained eggs were used in lab and greenhouse experiments right away.

2.2.4 Soybean cyst nematode egg hatching test:

A 96-well flat bottom microtiter plate (Falcon® 353072 MICROTTEST™ 96 Tissue Culture Plate; Becton Dickinson Labware; Franklin Lakes, NJ) was used for SCN egg hatching test. Fifty SCN eggs in 15 μ l double distilled water were added to the 235 μ l of plant extracts with two different concentrations per well in one set of the experiment. Double distilled water (ddH₂O) was used as negative control and 5mM ZnSO₄ was used as a positive control (Tefft and Bone 1984). Plant extracts were serially diluted between 94% and 2.5% using ddH₂O. In total, six concentrations of each plant extracts were tested in SCN egg hatching experiment, but only two concentrations were used at a time. Three sets of SCN egg hatching experiments were conducted comparing two concentrations, 94% and 50%, 25% and 12.5%, 5% and 2.5%. Plant extracts of 94% concentration is equivalent to 94 mg dry plant material in 1ml ddH₂O. Likewise, 2.5% plant extract is equivalent to 2.5 mg dry plant materials in 1ml of ddH₂O. Experimental design used was completely randomized block design with six replications. The microtiter plate was kept in an incubator at 25° C and number of juveniles hatched from the eggs were recorded each day after application of treatments up to 15 days. Counting of hatched juveniles was done under an inverted microscope (Nikon Eclipse Ts2). Number of juveniles hatched were converted into percentage hatched [(Number of eggs hatched per well/ 50)100%] for each day. The experiment was repeated once.

2.2.5 Soybean cyst nematode second stage juvenile (J2) mortality test:

SCN eggs were incubated at 25° C to encourage hatching and the second stage juveniles obtained were used in J2 mortality experiment. J2 mortality test followed the same procedures as egg hatching test, except eggs were replaced by 30 J2s per well. Number of dead J2s were recorded at every 12 hours interval. J2s in each well were probed with fine metal needle. When J2s did not respond to the needle probing, 2.5 μ l of one normal sodium carbonate (Na_2CO_3) was applied per well to confirm the mortality (Xiang and Lawrence 2016). Number of J2s dead were converted into mortality percentage J2s dead [(Number of J2s dead per well/30)100%] for every 12 hours interval. The experiment was repeated once.

2.2.6 Soybean cyst nematode female development test in greenhouse

Effect of plant extracts on the reproduction of SCN was carried out in greenhouse conditions. Soil from the SCN infested soybean field (Brookings county) was collected for the experiment. Thus, obtained soil was mixed well and SCN eggs and juveniles were counted and found to be around 4,000 per 100 cc of soil. Fifty-centimeter cube (cc) of field soil with SCN was mixed with 50 cc of sand to adjust the SCN density 2,000 eggs and juveniles per 100 cc of soil. Soybean variety susceptible to SCN, Williams 82 was used in this experiment. Pre-germinated soybean seeds were planted on the Cone tainer (3.81-cm diam. and 20.955-cm high; Stuewe and Sons, Inc., Tangent OR) filled with premixed soil with SCN. Plant extract of each plant material with two different concentrations, 100% (100 mg/ml) and 50% (50 mg/ml) was applied per plant (based on the in-vitro study). Total 75 ml plant extract was applied per plant at three split doses each at 25 ml of each extract.

First application of extract was done at the time of planting of soybean. Second and third application were done 7 and 14 days after planting (DAP), respectively. Tap water was used as the negative control and while ILeVO (commercial nematicide) treated seed was used as positive control in this experiment. Cones were placed in the 5-gallon bucket filled with sand and buckets were kept in the water bath. All the treatments (12 cones) were fitted in a bucket and each bucket was considered as a block. Experimental design was completely randomized block design with six replications. Temperature of water bath was maintained 27° C throughout the growing period with 16 hours of day light. Plants were watered every two days. Thirty days after planting, cones were taken out of the bucket and plant shoots were excised. Plant height and fresh plant shoot weight of each plant was recorded. Cones were dipped in the water for about 10 minutes for the ease of SCN cyst extraction. Each plant was uprooted, and roots were sprayed with a strong stream of water to dislodge the cysts. The cysts were collected in a 250- μm sieve nested under a 710- μm sieve and counted under the dissecting microscope. Root length and fresh root weight of each plant was measured and recorded. Cysts were crushed by using motorized rubber stopper to release eggs, and eggs were collected in 25 μm sieve nested under 75 μm sieve (Faghihi and Ferris 2000). Total number of eggs were counted under an inverted microscope (Nikon Eclipse Ts2, Company name and city and state). The experiment was repeated once.

2.2.7 Data analysis:

Data analysis was done using R studio version 3.5.3 (The R Foundation for Statistical Computing Platform). Total number of females and eggs per plant were standardized to per gram root mass before analysis. Analysis of variance (ANOVA) was used to determine

the effect of treatments on hatching of eggs, mortality of J2s, SCN reproduction, and plant growth parameters. Least significant difference (LSD) test was used to separate the means of the treatment at $P \leq 0.05$ using R package “Agricolae”

2.3 Results

Soybean cyst nematode egg hatching: All plant extracts up to 12.5% concentration (12.5 mg/ml) completely inhibited the egg hatching. There was limited egg hatching in the 5% (5mg/ml) and 2.5% (2.5mg/ml) plant extract treatments. Based on the Bartlett’s homogeneity test at $P \leq 0.05$, variances were not homogeneous for two experimental runs. Therefore, data for each run were analyzed separately. Overall egg hatching was higher in first run than the second run. In both runs, plant extract significantly inhibited the SCN egg hatching in comparison to ddH₂O and 5mMZnSO₄ on day 3, day 7 and day 15 (Table 2.1 and Table 2.2).

In experimental run 1, On day 3, the 5% plant extract concentrations of wormwood dry leaf and ragweed dry leaf significantly reduced the hatching than the 2.5% concentration. The rest of the plant extracts did not exhibit significant difference between two levels of concentrations. On the days 7 and 15, there was significant difference between two levels of each of the plant extracts on egg hatching (Table 2.1). On the day 15, average percentage eggs hatched was least in ragweed dry flower 5% (9.6%) and maximum on wormwood dry leaf 2.5% (28.3%) (Table 2.1). Among the plant extracts on day 3, 7, and 15, percentage eggs hatching in wormwood dry flower 5% was 0, 1, and 3%, respectively, which was least among the treatments.

Similarly in the experimental run 2, among the plant extracts on day 3, 7, and 15, percentage hatched was highest for ragweed dry leaf 2.5% (5%), ragweed dry flower 2.5% (9%), and wormwood dry leaf 2.5% (11%), respectively (Table 2.2). On the day 15, hatching was significantly lower at 5% concentration than the 2.5%, for ragweed dry flower, wormwood dry flower, and wormwood dry leaf (Table 2.2).

Soybean cyst nematode second stage juvenile (J2) mortality test: Based on the Bartlett's homogeneity test at $P \leq 0.05$, variances were not homogeneous for the two experimental runs. Therefore, data for each run were analyzed separately. Percentage J2s dead were compared at 84 hours after application of plant extracts. Treatments were significantly different in comparison to the control (ddH₂O). Overall percentage J2s mortality was lower in first run than the second in all the treatments. Among the plant extracts there was significant difference in percentage J2s mortality, while there was no J2s mortality in the control (ddH₂O) at 84 hours (Table 2.3). In first run at 84 hours after application of treatments, ragweed dry flower 94% (94 mg/ml) caused highest J2s mortality, 93.3%, while leafy spurge dry leaf 50% (50 mg/ml) caused lowest J2s mortality, 36.6% (Table 2.3). Plant extracts of wormwood dry flower, ragweed dry leaf, wormwood dry leaf, and leafy spurge dry leaf at 94% concentration caused 81.2%, 72.2%, 68.9%, and 49.4% J2 mortality, respectively at 84 hours after application of treatments (Table 2.3). Similarly, the 50% plant extract concentrations of ragweed dry leaf, wormwood dry flower, ragweed dry leaf, and wormwood dry leaf caused 61.7%, 52.2%, 50%, and 48.3% J2 mortality, respectively at 84 hours after application of treatments (Table 2.3). Cumulative percentage J2 mortality by application of 94% plant extracts across different time interval in experimental run 1 is shown in Figure 2.2.

In second run at 84 hours after application of treatments, ragweed dry flower 94% caused 100% J2s mortality, while leafy spurge dry leaf 50% caused lowest J2s mortality, 51.1% (Table 2.3). Plant extracts of wormwood dry flower, ragweed dry leaf, wormwood dry leaf, and leafy spurge dry leaf at 94% concentration caused 90.6%, 89.4%, 86.6%, and 57.2% J2 mortality, respectively after 84 hours of application of treatments (Table 2.3). Similarly, 50% plant extracts of ragweed dry leaf, wormwood dry flower, ragweed dry leaf, and wormwood dry leaf caused 76.6%, 73.3%, 74.4%, and 66.6% J2 mortality respectively at 84 hours after application of treatments (Table 2.3). Cumulative percentage J2 mortality by application of 94% plant extracts across different time interval in experimental run 2 is shown in Figure 2.3.

The order of percentage J2s mortality at 84 hours after application of treatment in both runs was, ragweed dry flower > wormwood dry flower > ragweed dry leaf > wormwood dry leaf > leafy spurge.

Soybean cyst nematode female development test in greenhouse: In both runs, plant extract from dry plant materials significantly reduced the reproduction of SCN in comparison to the control (Tap water) in the greenhouse conditions. Two runs were significantly different and there was significant interaction between plant extracts and experimental runs. So, each run was analyzed separately.

SCN cysts per plant: Plant extracts greatly reduced the total cysts in the soybean root in both runs. Effect of plant extracts on the total number of cysts (log transformed) per plant is shown in Figure 2.4 and Figure 2.5 for run 1 and 2, respectively. In both runs, 100% extract of ragweed dry flower reduced the number of cysts per plant as effectively as

ILeVO. Wormwood dry flower 100% also reduced the total cysts per plant like the ILeVO in the second run. The order of reduction of total cysts per plant by plant extracts for the first run was, ragweed dry flower > ragweed dry leaf > wormwood dry flower > wormwood dry leaf > Leafy spurge. In the second run, wormwood dry flower and leaf reduced the total cysts per plant greatly after ragweed dry flower. Leafy spurge had the least effect on the total number of cysts in both runs.

SCN cysts per gram of root: In both runs, all the plant extracts and positive control (Chemical nematicide seed treatment- ILeVO) significantly reduced the number of cysts per gram of root in comparison to the control (Tap water) in the greenhouse conditions (Table 2.4 and Table 2.6). In run 1, among the plant extracts, both concentrations of ragweed dry flower (100% and 50%) and ragweed dry leaf 100% were as effective as ILeVO seed treatment (Table 2.4). In the second run, 100% plant extracts of ragweed dry flower, ragweed dry leaf, and wormwood dry leaf were not significantly different with ILeVO seed treatment (Table 2.6). In run 1, based on the mean separation with least significant difference (at $\alpha = 0.05$), effect of two levels (100% and 50%) of each plant extract on the average number of SCN females per gram of root was significantly different except for the leafy spurge dry leaf, whereas, only ragweed dry flower and wormwood dry leaf were significantly different in the second run (Table 2.4 and Table 2.6). Among the plant extracts the order of reduction in average number of SCN cysts per gram of root was, ragweed dry flower > ragweed dry leaf > wormwood dry leaf > wormwood dry flower > leafy spurge dry leaf (Table 2.4). In the run 2, order was same except for the ragweed dry leaf and wormwood dry leaf, where reduction of females per gram of root was higher for wormwood dry leaf than the ragweed dry leaf (Table 2.6).

Total eggs per gram of root: In both runs, all the plant extracts and positive control (Chemical nematicide seed treatment- ILeVO) significantly reduced the average number of eggs per gram of root mass in comparison to the control (Tap water) in the greenhouse conditions (Table 2.4 and Table 2.6). In run 1, the trend and order for the reduction of eggs due to the plant extract was like the effect of plant extracts on average females per gram of root except for the ragweed dry leaf 100%, which significantly decreased the average number of eggs in comparison to the ILeVO seed treatment (Table 2.4). In run 2, both flower and leaf extracts from ragweed and wormwood at 100% concentration reduced the SCN eggs per gram of root as effectively as ILeVO seed treatment (Table 2.6). For the rest of the extracts, effect was like on the SCN females per gram of root (Table 2.6).

Eggs per female: In first run, eggs per female was significantly lower in the plant extracts in comparison to both controls (Table 2.4). Among the plant extracts there was no significant difference on the average number of eggs per female. In second run, average number of eggs per female was significantly lower in the plant extracts in comparison to both controls except for the leafy spurge dry leaf (100 and 50%) and ragweed dry leaf 50% (Table 2.6).

Effect of plant extract on the plant growth parameters: In both runs, plant extracts significantly affected the plant height, fresh plant shoot weight, root length, and fresh root weight in comparison to the control.

Plant height: In run 1, only leafy spurge dry leaf 100% significantly increased the plant height (23.13 cm) in comparison to the control (21.37 cm) (Table 2.5). Plants treated with leafy spurge dry leaf 50% and wormwood dry leaf 50% did not affect the plant height in

comparison to the control. The rest of the treatments including positive control (Chemical nematicide seed treatment- ILeVO) significantly reduced the plant height in comparison to the control (Tap water) (Table 2.5). In run 2, both concentrations (100% and 50%) of leafy spurge dry and ragweed dry leaf 50% significantly increased the plant height in comparison to the control (Table 2.7). ILeVO seed treatment and both concentrations of wormwood dry flower significantly reduced the plant height in comparison to the control. The rest of the treatments did not affect the plant height in comparison to the control (Table 2.7).

Fresh plant shoot weight: In the first run, only the leafy spurge dry leaf 100% significantly increased the fresh plant shoot weight (5.1 gm) in comparison to the control (3.1 gm) (Table 2.6). The rest of the treatments did not significantly affect the fresh plant shoot weight (Table 2.6). In run 2, plants treated with wormwood dry flower 50% (2.4 gm) and wormwood dry flower 100% (2.6 gm) significantly reduced the fresh plant shoot weight in comparison to the control (3.4 gm) (Table 2.7). The rest of the treatments did not significantly affect the fresh plant shoot weight (Table 2.7).

Root length: In first run, there was no significant effect of treatments on the average root length of soybean plants in comparison to control. In second run, both concentrations of ragweed dry leaf, and leafy spurge significantly increased the root length in comparison to the control (21.41 cm) (Table 2.7). Wormwood dry leaf and ILeVO did not affect the root length in comparison to the control, whereas wormwood dry flower significantly reduced the root length (Table 2.7).

Fresh root weight: In the first run, average fresh root weight in wormwood dry leaf 100%, leafy spurge dry leaf 100%, and wormwood dry leaf 50% was 3.1 gm, 2.71 gm, and 2.6

gm, respectively, which was significantly higher than control (1.8 gm) (Table 2.5). Ragweed dry leaf 100% (1.4 gm), ragweed dry flower 100% (1.4), and ILeVO (1.358) significantly reduced the root weight in comparison to the control (Table 2.5). The rest of the treatments did not affect the fresh root weight of the plants in comparison to the control (Table 2.5). In the second run, both concentrations (100% and 50%) of plant extract from ragweed dry leaf, wormwood dry leaf, leafy spurge dry leaf, and ragweed dry flower 50% significantly increased the fresh root in comparison to the control (2.1 gm) (Table 2.7). But the wormwood dry flower 100% significantly reduced the fresh root weight in comparison to the control, where the average root weight was 1.69 gm (Table 2.7). ILeVO, ragweed dry flower 100%, and wormwood dry flower 50%, and did not affect the fresh root weight in comparison to the control (Table 2.7).

2.4 Discussion

Both lab and greenhouse experiment results show that all the plant materials tested against SCN development possessed nematicidal properties. Plant extracts significantly inhibited the egg hatching and caused second stage juvenile (J2) mortality in the lab experiments and significantly reduced the SCN reproduction in greenhouse conditions.

Plant extracts greatly inhibited the hatching for the first 3-4 days and hatching continued up to 12th day whereas, in ddH₂O and 5 mM ZnSO₄ hatching continued up to 15th day. In the first run ragweed dry flower 5mg/ml inhibited SCN egg hatching by 90.33% whereas, wormwood dry flower 5mg/ml inhibited hatching by 97% in the second run. The order of hatching inhibition was not consistent across the days and experimental runs. We observed the inhibition on SCN egg hatching due to the application of plant extracts, but the

mechanism is unknown. Similar levels of egg hatch inhibitory effect of 0.5% and 0.25% essential oils of *Artemisia absinthium* was reported on *Meloidogyne incognita* (Amora et al. 2017).

Based on the J2 mortality, ragweed dry flower and wormwood dry flower at the concentration of 94 mg/ml were found to be the most effective in both experimental runs. We found the variation in defining the mortality of J2s among the different independent studies. In an experiment of J2 mortality of *Meloidogyne incognita* with different extracts of *Artemisia absinthium*, non-motile juvenile with straight body was considered dead even if that responded the fine needle probing Liu et al. (2019). Zhou et al. (2017) probed the J2 with fine needle and considered dead if that did not respond the probing. In our experiment at first, we used fine needle and when J2s stopped responding needle probing we applied 2.5 μ l per well at pH 10 to distinguish live and dead J2 (Xiang and Lawrence 2016).

Similar J2 mortality caused by various extracts and essential oils of wormwood were reported on *Meloidogyne incognita* (Liu et al. 2019) and *Meloidogyne javanica* (Amora et al. 2017). Percentage J2 mortality was significantly lowest in leafy spurge dry leaf extract in comparison to the other plant extracts. Similar J2 mortality effect of ethanolic extract of leafy spurge was reported on (Liu et al. 2014), where the percentage J2 mortality was 39.9%.

There was significant reduction in average number of SCN cysts and eggs per gram of root mass due to the application of plant extracts in both runs. Ragweed dry flower and dry leaf at 100% concentration consistently reduced the average number of SCN females per gram

of root as effectively as ILeVO seed treatment in both runs. Effect of plant extracts on average number SCN females per gram of root was dose (100% and 50%) dependent for ragweed dry flower and wormwood dry leaf for both runs. There was no significant effect of dose for leafy spurge, while ragweed dry leaf and wormwood dry leaf were showing dose dependent effect only in first run. Contradictory result of essential oils of wormwood on the different plant parasitic nematode- *Meloidogyne javanica* has been reported by Amora et al. (2017), where gall formation by the nematode in tomato plant was increased. Similar result of decrease in population of *Rotylenchus robustus* (Thorne's lance nematode) by ragweed in the field was reported previously (Gommers 1973).

Our experiment was focused on the investigation of nematicidal properties of three plant species known to affect various plant parasitic nematodes. The next step will be investigating the chemical composition in these extracts that are demonstrating nematicidal properties. Oka (2010) explained the increase in plant tolerance and resistance as a mechanism of nematode suppression with organic soil amendments this might be similar with SCN suppressive nature of plant extracts. Julio et al. (2017) reported that wormwood contains (5Z)-2,6-dimethylocta-5,7-diene-2,3-diol compound, which is the potential nematode control agent. Nematicidal property of *Ambrosia artemisiifolia* and *Euphorbia esula* has not been well studied yet. High content of sesquiterpene lactones in ragweed plants are reported to have antihelminthic property in which cumanin and peruvins are the main sesquiterpene lactones found in above ground parts (Parkhomenko et al. 2005). These compounds might be responsible for the nematicidal property of ragweed. Leafy spurge contains diterpenoid ingenols esters (Halaweish et al. 2002), jatrophone diterpenoid esters (Liu et al. 2002), and lathyrane diterpenoid esters (Onwukaeme and Rowan 1992), these

toxic compounds might be showing the nematicidal effect in the extract prepared from leafy spurge.

Plant extracts also significantly affected the plant growth parameters (plant height, fresh plant shoot weight, root length, and fresh root weight) in comparison to the control check. Plant extracts from leafy spurge and ragweed dry leaf not only inhibited the SCN reproduction in the greenhouse but also significantly increased the plant height. Similar case of increase in plant height by the application of plant extracts prepared from acacia (*Acacia nilotica*), neem (*Azadirachta indica*), Chinese cabbage (*Brassica chinensis*), and sea bamboo (*Ecklonia maxima*) has been reported by Auwal et al. (2013). In contrast, ILeVO, ragweed, wormwood dry flower, and wormwood dry leaf at 100% significantly reduced the plant height in comparison to the control in the first run, while only ILeVO and Wormwood dry leaf significantly reduced the plant height in the second run. Similar results of negative effect on plant growth parameters due to the application of plant-based products has been reported by Grabau (2013). Negative effect of the plant extracts on the soybean plant growth parameters might be due to the possible phytotoxicity effects. Phytotoxicity of ILeVO seed treatment on soybean seedling was observed in early stage with signs of yellow-brown color on the tips of the cotyledons as described by Wise et al. (2015), which might be the possible explanation for negative effect of ILeVO on plant growth parameters of soybean plants treated with ILeVO. Decrease in root weight in ILeVO treated soybean plants has been reported by Beeman and Tylka (2018).

Negative effect of plant extracts on the plant growth parameters of soybean plants observed in our experiments might be due to the phytotoxicity of the chemicals present in the plant extracts. From this experiment we cannot make conclusion whether the same chemical

compounds are responsible for nematicidal property of the plant extracts and phytotoxicity effect. Further experiment is required for the possible explanation of both positive and negative effect on plant growth parameters of soybean plants, but there is no doubt that all the plant materials tested in this experiment have nematicidal property in varying degree on the basis of egg hatch, J2 mortality and female development test in the greenhouse.

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Table 2.1. Cumulative percentage *Heterodera glycines* eggs hatched on 3, 7, and 15 days after application of plant extracts at the concentration of 5% (5 mg/ml) and 2.5% (2.5 mg/ml) under laboratory conditions, experimental run 1

Treatments	Cumulative egg hatch (%)		
	Day 3	Day 7	Day 15
Wormwood dry flower 5%	0.33 e	6.67 fg	15.33 defg
Ragweed dry flower 5%	0 e	5.33 e	9.67 g
Wormwood dry leaf 5%	0.33 e	4.67 g	14 efg
Ragweed dry leaf 5%	1 de	11 def	20 cdef
Leafy spurge dry leaf 5%	1.33 cde	5.33 fg	12 fg
Wormwood dry flower 2.5%	1.67 cde	12 de	25.67 c
Ragweed dry flower 2.5%	2 cde	12.67 d	22.67 cde
Wormwood dry leaf 2.5%	3 cd	19.67 c	28.33 c
Ragweed dry leaf 2.5%	3.67 c	13.67 d	22 cde
Leafy spurge dry leaf 2.5%	1.67 cde	12.67 d	23 cd
5 mM ZnSO ₄	16 a	45.33 a	80 a
ddH ₂ O	12.67 b	39 b	58 b

Each value is mean of 6 replications. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 2.2. Cumulative percentage *Heterodera glycines* eggs hatched on 3, 7, and 15 days after application of plant extracts at the concentration of 5% (5mg/ml) and 2.5% (2.5 mg/ml) under laboratory conditions, experimental run 2

Treatments	Cumulative egg hatch (%)		
	Day 3	Day 7	Day 15
Wormwood dry flower 5%	0 e	1 e	3 f
Ragweed dry flower 5%	0.67 de	1.67 e	4 ef
Wormwood dry leaf 5%	1.67 de	3.33 de	3.67 ef
Ragweed dry leaf 5%	1 de	3.67 cde	4.67 def
Leafy spurge dry leaf 5%	1 de	4 cde	5 cdef
Wormwood dry flower 2.5%	2.33 cde	7.66 cd	9.33 cde
Ragweed dry flower 2.5%	3.67 cd	9 c	10.33 cd
Wormwood dry leaf 2.5%	1.67 de	7.33 cd	11 c
Ragweed dry leaf 2.5%	5 c	8.67 cd	9.67 cde
Leafy spurge dry leaf 2.5%	1 de	5.67 cde	9.33 cde
5 mM ZnSO ₄	26.33 a	52.67 a	65.67 a
ddH ₂ O	17 b	40.33 b	52.33 b

Each value is mean of 6 replications. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 2.3. Cumulative percentage *Heterodera glycines* second stage juvenile (J2) mortality at 84 hours after the application of treatments at the concentration of 94% (mg/ml) and 50% (5 mg/ml) under laboratory conditions, experimental run 1 and run 2

Treatments	Cumulative J2 mortality (%)	
	Run 1	Run 2
Wormwood dry flower 94%	81.17 ab	90.55 ab
Ragweed dry flower 94%	93.33 a	100 a
Wormwood dry leaf 94%	68.89 bc	86.67 bc
Ragweed dry leaf 94%	72.23 bc	89.44 b
Leafy spurge dry leaf 94%	49.44 def	57.22 ef
Wormwood dry flower 50%	52.22 de	73.33 d
Ragweed dry flower 50%	61.67 cd	76.67 cd
Wormwood dry leaf 50%	48.33 ef	66.67 de
Ragweed dry leaf 50%	50 de	74.44 d
Leafy spurge dry leaf 50%	36.67 f	51.11 f
Double distilled water	0 g	0 g

Each value is mean of 6 replications. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 2.4. Effect of 100% (100 mg/ml) and 50% (50 mg/ml) plant extracts on the reproduction of *Heterodera glycines* after 30 days of treatments using field soil (2,000 eggs/100 cc soil) in the greenhouse conditions, experimental run 1

Treatments	Cyst per gram of root	Eggs per gram of root	Eggs per cyst
Wormwood dry flower 100%	2.57 ef	8.21 ef	280.19 c
Ragweed dry flower 100%	1.44 i	7.08 h	278.5 c
Wormwood dry leaf 100%	2.41 efg	8.07 ef	286.96 bc
Ragweed dry leaf 100%	2.26 fgh	7.92 fg	288.49 bc
Leafy spurge dry leaf 100%	3.15 cd	8.83 cd	293.63 bc
Wormwood dry flower 50%	3.6 b	9.26 bc	287.74 bc
Ragweed dry flower 50%	2.1 gh	7.73 fg	279.3 c
Wormwood dry leaf 50%	3.83 b	9.5 b	289.2 bc
Ragweed dry leaf 50%	2.78 de	8.44 de	288.89 bc
Leafy spurge dry leaf 50%	3.51 bc	9.18 bc	291.71 bc
ILeVO seed treatment	1.86 hi	7.57 g	301.15 ab
Tap water	4.84 a	10.58 a	313.02 a

Each value is mean of 6 replications. Value for female per gram of root and eggs per gram root were log transformed. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 2.5. Effect of 100% (100 mg/ml) and 50% (50 mg/ml) plant extracts on the growth of soybean after 30 days of treatments using field soil (2,000 eggs/100 cc soil) in the greenhouse conditions, experimental run 1

Treatments	Plant height (cm)	Fresh plant shoot weight (gm)	Fresh root weight (gm)
Wormwood dry flower 100%	17.67 fg	3.37 cd	1.63 de
Ragweed dry flower 100%	16.8 gh	2.36 fg	1.36 e
Wormwood dry leaf 100%	18.8 def	4.6 b	3.05 a
Ragweed dry leaf 100%	18.83 def	2.22 d	1.43 e
Leafy spurge dry leaf 100%	23.13 a	5.09 a	2.71 ab
Wormwood dry flower 50%	18.3 efg	3.67 c	2.18 c
Ragweed dry flower 50%	19.65 cde	2.74 ef	1.57 de
Wormwood dry leaf 50%	20.1 bcd	4.33 b	2.61 b
Ragweed dry leaf 50%	19.48 de	2.43 fg	1.61 de
Leafy spurge dry leaf 50%	21.27 bc	3.64 c	2.04 c
ILeVO seed treatment	15.83 h	2.55 fg	1.36 e
Tap water	21.37 b	3.07 de	1.85 cd

Each value is mean of 6 replications. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 2.6. Effect of 100% (100 mg/ml) and 50% (50 mg/ml) plant extracts on the reproduction of *Heterodera glycines* after 30 days of treatments using field soil (2,000 eggs/100 cc soil) in the greenhouse conditions, experimental run 2

Treatments	Cyst per gram of root	Eggs per gram of root	Eggs per cyst
Wormwood dry flower 100%	3.46 bcde	9.12 bcde	285.64 de
Ragweed dry flower 100%	3.05 ef	8.7 e	283.55 e
Wormwood dry leaf 100%	3.27 def	8.94 de	290.46 bcde
Ragweed dry leaf 100%	3.33 cdef	8.99 cde	287.46 cde
Leafy spurge dry leaf 100%	3.86 b	9.57 b	300.8 abcd
Wormwood dry flower 50%	3.81 b	9.48 b	290.04 bcde
Ragweed dry flower 50%	3.57 bcd	9.24 bcd	291.52 bcde
Wormwood dry leaf 50%	3.77 bc	9.46 bc	295.17 bcde
Ragweed dry leaf 50%	3.65 bcd	9.35 bcd	297.92 abcde
Leafy spurge dry leaf 50%	3.86 b	9.59 b	306.15 ab
ILeVO seed treatment	2.97 f	8.68 e	302.44 abc
Tap water	5.04 a	10.78 a	311.77 a

Each value is mean of 6 replications. Value for female per gram of root and eggs per gram root were log transformed. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 2.7. Effect of 100% (100 mg/ml) and 50% (50 mg/ml) plant extracts on the growth of soybean after 30 days of treatments using field soil (2,000 eggs/100 cc soil) in the greenhouse conditions, experimental run 2

Treatments	Plant height (cm)	Fresh plant shoot weight (gm)	Root length (cm)	Fresh root weight (gm)
Wormwood dry flower 100%	16.85 g	2.61 d	20.03 d	1.688 f
Ragweed dry flower 100%	21.717 bcd	3.55 abc	22.467 ab	2.195 cde
Wormwood dry leaf 100%	20.05 ef	3.12 c	21.567 c	2.447 bc
Ragweed dry leaf 100%	21.20 cde	3.76 a	22.6 ab	2.994 a
Leafy spurge dry leaf 100%	23.083 a	3.76 a	22.75 a	2.698 ab
Wormwood dry flower 50%	13.833 h	2.48 d	20.583 d	2.208 cd
Ragweed dry flower 50%	22.067 abcd	3.68 ab	22.45 ab	2.417 bc
Wormwood dry leaf 50%	21.333 bcd	3.39 abc	21.867 bc	2.508 bc
Ragweed dry leaf 50%	22.517 ab	3.79 a	22.4 ab	2.717 ab
Leafy spurge dry leaf 50%	22.283 abc	3.77 a	22.633 ab	2.979 a
ILeVO seed treatment	19.45 f	3.29 bc	21.517 c	1.875 ef
Tap water	20.817 de	3.41 abc	21.417 c	2.079 de

Each value is mean of 6 replications. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test.



(A)



(B)



(C)

Figure 2.1. Wormwood (*Artemisia absinthium*) freshly harvested shoot (A), Ragweed (*Ambrosia artemisiifolia*) freshly harvested floral parts with leaves (B) and leafy spurge (*Euphorbia esula*) dry plant (C)

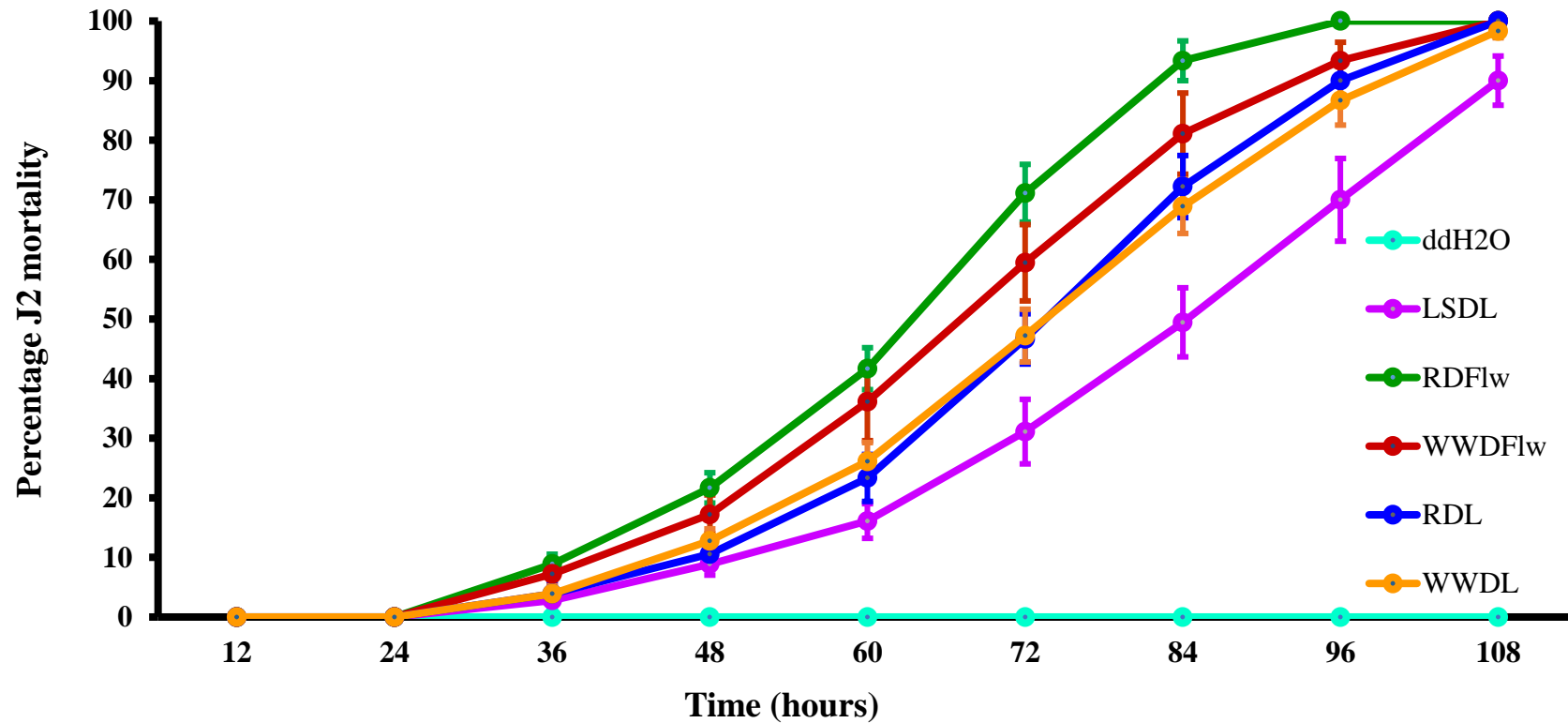


Figure 2.2. Percentage J2 mortality (mean \pm standard error of the mean) by plant extracts treatments at the concentration of 94% (94 mg/ml) across different time intervals, Experimental run 1. There were six replications.

ddH₂O: Double distilled water; LSDL: Leafy spurge dry leaf; RDFlw: Ragweed dry flower; RDL: Ragweed dry leaf; WWDFlw: Wormwood dry flower; WWDL: Wormwood dry leaf

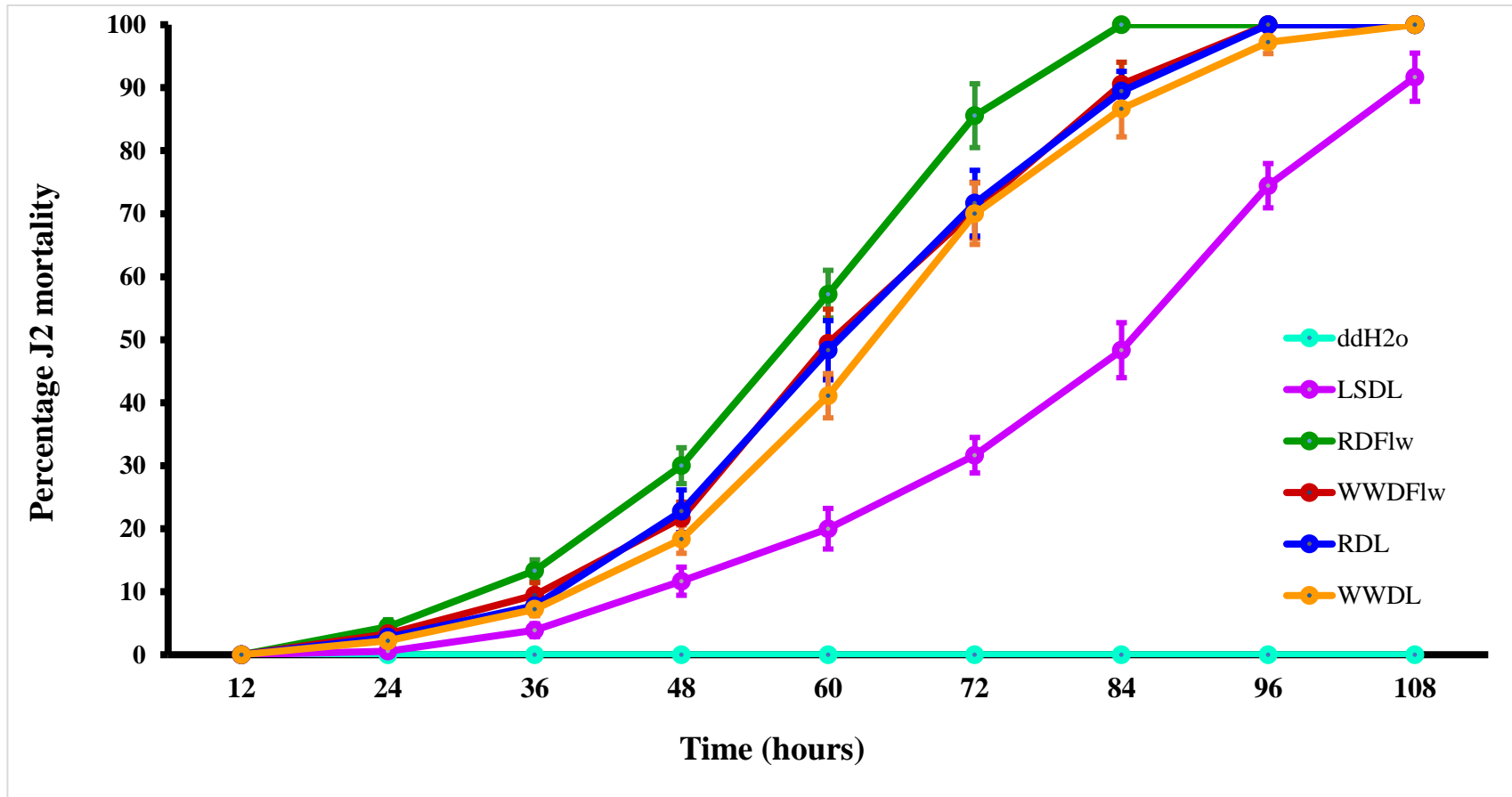


Figure 2.3. Percentage J2 mortality (mean \pm standard error of the mean) by plant extracts treatments at the concentration of 94% (94 mg/ml) across different time intervals, Experimental run 2. There were six replications.

ddH₂O: Double distilled water; LSDL: Leafy spurge dry leaf; RDFlw: Ragweed dry flower; RDL: Ragweed dry leaf; WWDFlw: Wormwood dry flower; WWDL: Wormwood dry leaf

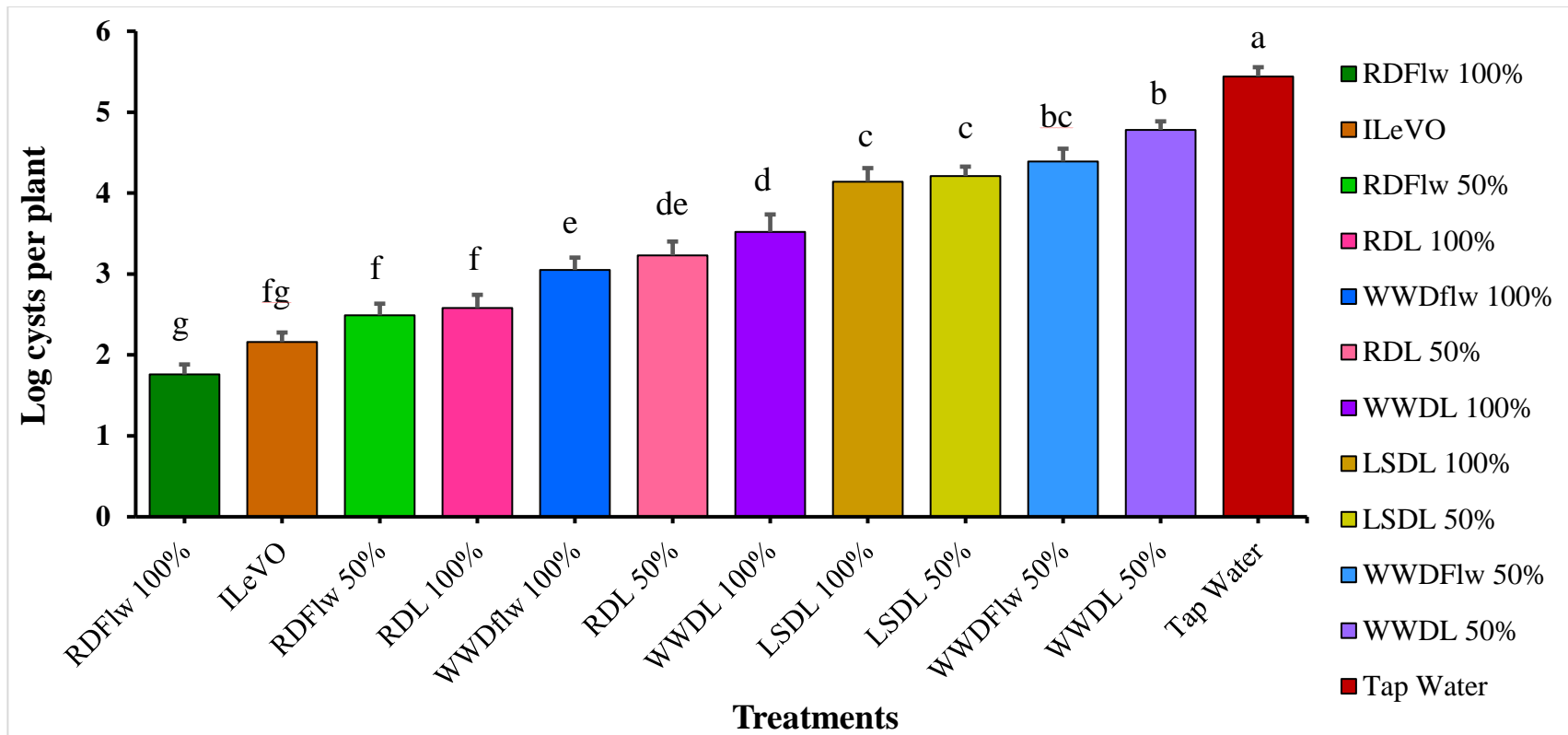


Figure 2.4. Effect of plant extracts at the concentration of 100% (100 mg/ml) and 50% (50 mg/ml) on the total number of *Heterodera glycines* cysts (mean \pm standard error of the mean) per plant in the greenhouse conditions, experimental run 1. There were six replications. Value for total number of cysts per plant was log transformed. Bars with same letters are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test.

RDFLW: Ragweed dry flower; RDL: Ragweed dry leaf; WWDFlw: Wormwood dry flower; WWDL: Wormwood dry leaf; LSDL: Leafy spurge dry leaf

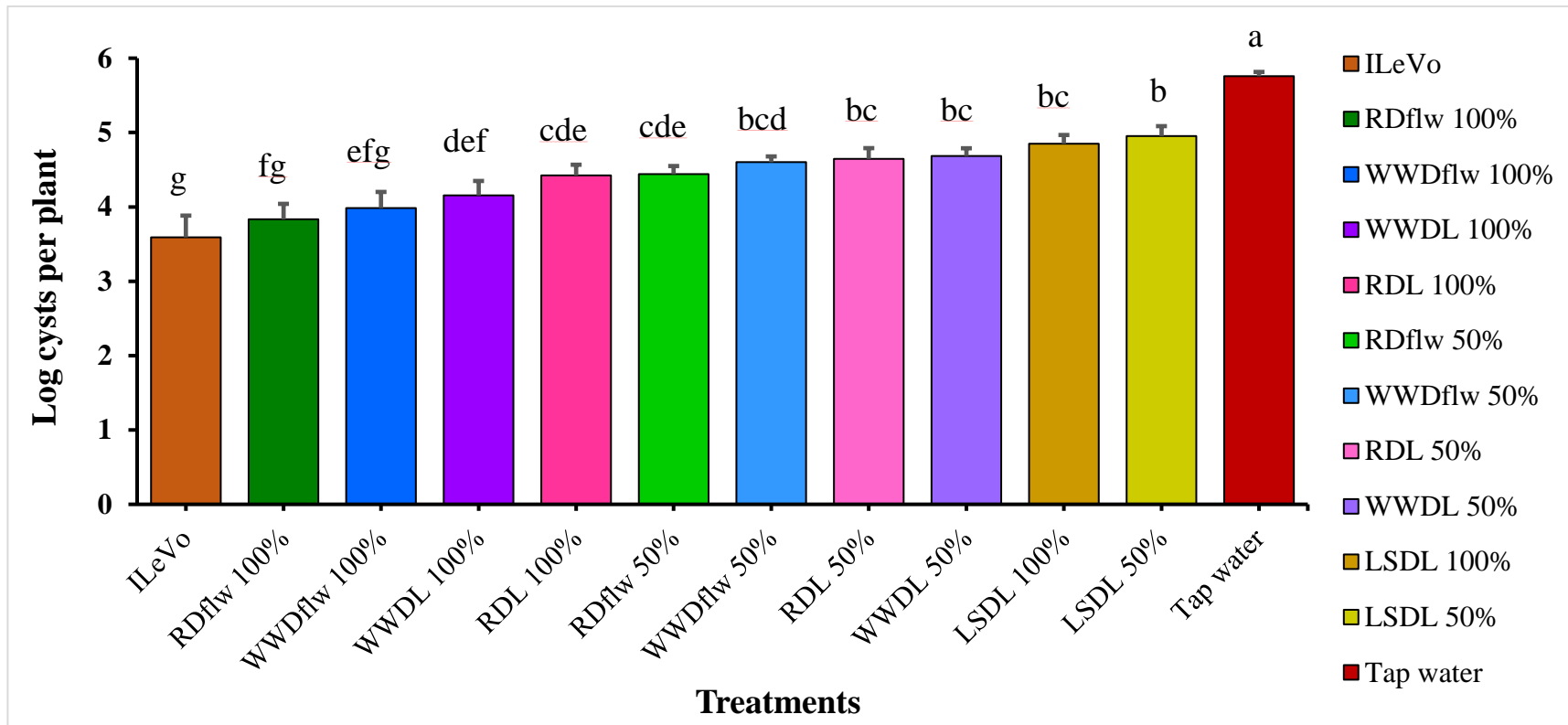


Figure 2.5. Effect of plant extracts at the concentration of 100% (100 mg/ml) and 50% (50 mg/ml) on the total number of *Heterodera glycines* cysts (mean \pm standard error of the mean) per plant in the greenhouse conditions, experimental run 2. There were six replications. Value for total number of cysts per plant was log transformed. Bars with same letters are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test.

RDFLW: Ragweed dry flower; RDL: Ragweed dry leaf; WWDFlw: Wormwood dry flower; WWDL: Wormwood dry leaf; LSDL: Leafy spurge dry leaf

Chapter 3

3 Determining the efficacy of Saltro chemical nematicide in the lab and greenhouse conditions

Abstract

Soybean cyst nematode (SCN) is a one of the major yield limiting destructive pathogen of soybean crop. SCN may cause up to 30% soybean yield loss without visible above ground symptoms. Nematicide seed treatment is becoming a popular management strategy because it is safer than field application of chemicals and can be applied in combination with fungicides and insecticides simultaneously on the seed. We tested the effect of new chemical nematicide Saltro in SCN egg hatching and reproduction in lab and greenhouse conditions, respectively. Seed treatment with IleVO was used as commercial check. Susceptible soybean cultivar Williams 82 was used in the experiments. Seed exudates collected from Saltro treated seed reduced the SCN egg hatching by 55.4% compared to ddH₂O, whereas radicle exudates reduced the egg hatching by 37.5%. In greenhouse condition, Saltro reduced the SCN cysts per plant by 90.5% at 30 days after planting (DAP) and by 80.3% at 60 DAP, compared to control. Saltro seed treatment reduces the SCN egg hatching and reproduction in comparison to the control. Our findings will be helpful to the researchers to do the further efficacy studies under field conditions and farmers to make the decision while selecting the nematicide seed treatments.

Key words: Soybean Cyst Nematode (SCN), Nematicide, Seed treatment

3.1 Introduction

Soybean [*Glycine max* (L.)] ranks second in United States in terms of production and acreage (USDA FAS, 2018-2019). Soybean is used as protein source for humans, and byproducts from processed soybean are used for animal feed, ink, biofuels, plastic, and lubricants (SoyStats2018). Soybean is second most grown crop, after corn in United States (USDA FAS, 2018-2019). Soybean cyst nematode is among important economically impacting pathogens in United States. Currently, SCN has been reported in 17 states in U.S and parts of Canada (Tylka and Marett 2017). In South Dakota (SD), SCN was first detected in 1995 in Union County (Smolik and Draper 1998) and has been found in 34 counties as of June 2020 (Byamukama E., unpublished). The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is an obligatory, sedentary, endo-parasitic, microscopic worm, which infects and feeds from the soybean roots, hence robbing the plant of nutrients (Ichinohe 1952). SCN entry wounds in the soybean root may facilitate other pathogens to infect the roots (Riggs 2004; Tabor et al. 2003). SCN also interacts with other pathogens of soybean and increases the severity of the diseases, hence causing more yield loss (Xing and Westphal 2006a; Xing and Westphal 2006b; Xing and Westphal 2013). SCN can cause up to 30% yield loss of soybean without noticeable above ground symptoms (Niblack and Tylka 2008).

Chemical control is one of the most widely used SCN management practice, because of its quick effect and availability in the market. In the past years, fumigant nematicides were used most extensively than the non-fumigants with both non-systemic and systemic properties (Andersen and Sarma 2002; Duniway 2002; Kabana 1992; Rich et al. 2004; Taylor 2003). Fumigants, effective to control SCN in the field are no more available due

to their negative effects (Haydock et al. 2013). Rather than soil application of nematicide, seed treatment is becoming a new management strategy because it is safer than field application and can be applied in combination with fungicides and insecticides simultaneously (Munkvold et al. 2014).

Seed treatment with diverse biological active ingredients are available for SCN management. Aveo EZ (*Bacillus amyloliquefaciens* PTA 4838) (Valent Bio Sciences), CLARIVA (*Pasteuria nishizawae*) (Syngenta), VOTiVO (*Bacillus firmus* I-1582) (Bayer Crop Science) are common commercial biological formulations for seed treatment targeted to manage SCN. Chemical seed treatments such as ILeVO (AI: Fluopyram) (Bayer Crop Science), and AVICTA (AI: abamectin) (Syngenta) are commonly used.

Katsande (2019) studied the effect of seed treatment using biological formulations of *Bacillus firmus* and *Bacillus amyloliquefaciens*, and chemical nematicide, fluopyram (ILeVO) on the dry beans against SCN in controlled environment and field conditions. Effect of seed treatment on the SCN reproduction was less in the field conditions than the controlled environment and inconsistent across the dry bean cultivars. Although seed treatment has been widely used as a SCN management strategy, the effectiveness and consistency are still in question. Abamectin seed treatment was not able to suppress nematode (*M. incognita* and *R. reniformis*) in later growth stages of cotton. In addition, the transfer of nematicide to the growing tissues was also very low (Faske and Starr 2007). Similarly, the nematicide seed treatment was less effective than soil application in cotton (Wheeler et al. 2013), whereas no significant increase in yield was seen over years on treated Soybean compared to control (Gaspar et al. 2014). No consistent effects of ILeVO seed treatment on SCN egg hatching and J2 mortality were found under controlled

environment (Beeman and Tylka 2018). These inconsistent results observed regarding the effectiveness of commercial nematicides tested indicate that thorough testing of new nematicides in the market is necessary to assure its relative effectiveness compared to others.

In this study, we tested new chemical nematicide, Saltro (pydiflumetofen: Syngenta), for its efficacy on SCN egg hatching and reproduction. The finding of this study will help farmers in decision making process for choosing an appropriate nematicide, as per their requirement.

3.2 Materials and methods

3.2.1 Seed treatment

Soybean cyst nematode susceptible cultivar Williams 82 seeds were treated with Saltro (Pydiflumetofen 41.7%) 15.08 μ l/100 seeds (Syngenta). To compare performance of Saltro with a common seed treatment nematicide, ILeVO (Fluopyram 48.4%) 28.57 μ l/100 seeds (Bayer CropScience) was included in the experiment as a commercial check. Both Saltro and ILeVO treated seeds were used by collecting seed and radicle exudates and testing these exudates on egg and juvenile mortality and by planting nematicide treated seeds in the greenhouse and determining cyst development.

3.2.2 Soybean cyst nematode inoculum

Soybean variety Williams 82 was used to multiply and maintain the soybean cyst nematode in the greenhouse required for the lab and greenhouse experiments. SCN cysts (dead females) were extracted from 4-8 weeks old plants. Each plant was uprooted, and root was

sprayed with a strong stream of water to dislodge the SCN cysts. SCN cysts were collected in a 250- μm sieve nested under a 710- μm sieve. Cysts were surface sterilized with 0.5% NaOCl for 3 minutes and rinsed with double distilled water for three times (Hu et al. 2018) and crushed by using motorized rubber stopper to release eggs, and eggs were collected in 25 μm sieve nested under 75 μm sieve (Faghihi and Ferris 2000). Thus, obtained eggs were used in lab and greenhouse experiments.

3.2.3 Collection of seed and radicle exudates

To determine the efficacy of Saltrao seed treatment on SCN mortality, seed and radicle exudates were collected from the chemical nematicides (Saltro and ILeVO) treated seeds for in vitro SCN egg hatching experiments, whereas untreated seeds were used for the control. Seed and radicle exudates were collected following the method used in previous studies (Beeman and Tylka 2018; Riga et al. 2005; Zhao et al. 2000). Briefly, seeds were soaked in double distilled water (one seed per 5ml ddH₂O) in a 50 ml beaker and kept at 25° C for 48 hours to collect the seed exudates. Soaked seeds were agitated for one hour at 100 rpm on a platform shaker. Exudate was first centrifuged (BECKMAN COULTER; Avanti JXN-30) at 10,000xg for 20 minutes and filtered through syringe filter (Millex® Syringe Filter Units, Sterile, 33mm). For the radicle exudate, seeds were germinated on petri-dish with 1.5% water agar and petri-dish was incubated at 25° C in dark. When the radicles were 5-7 cm long, germinated seeds were suspended on the fine nylon mesh while radicles were dipped in the 50 ml beaker containing ddH₂O (one radicle per 5ml ddH₂O). Beaker was agitated on the platform shaker for an hour and radicle exudate was centrifuged and filtered as above.

3.2.4 Soybean cyst nematode egg hatching test:

Brown SCN cysts were hand-picked, and surface sterilized with 0.5% NaOCl for 3 minutes and rinsed with double distilled water for three times (Hu et al. 2018). Cysts were crushed by using motorized rubber stopper and eggs were collected in 25 μm sieve (Faghihi and Ferris 2000). 96 well flat bottom plate (Falcon® 353072 MICROTTEST™ 96 Tissue Culture Plate; Becton Dickinson Labware) was used for SCN egg hatching test. 50 SCN eggs in 15 μl double distilled water were added to the 235 μl of seed and radicle exudates per well. Seed exudate from non-treated seed was used as a control. Double distilled water (ddH₂O) was used as negative control and 5mMZnSO₄ was used as a positive control (Tefft et al. 1982). Treatments were laid out in a completely randomized block design with six replications. The plates were kept inside the incubator at 25° C and number of juveniles hatched from the eggs per well were recorded each day after application of treatments up to 15th day. Number of eggs hatched were converted into percentage hatched for each day as follows: Percentage egg hatched = (Total number of eggs hatched per well/50) x100%
The experiment was repeated once.

3.2.5 Soybean cyst nematode female development test in greenhouse

Effect of Saltro nematicide seed treatment on the reproduction of SCN was carried out in greenhouse conditions. Soil from the SCN infested soybean field (Brookings county) was collected for the experiment. Thus, obtained soil was mixed well and SCN density was adjusted (maintained) 2,000 eggs and juveniles per 100 cc of soil. Soybean variety susceptible to SCN, Williams 82, was used in this experiment. Pre-germinated, Saltro nematicide treated soybean seeds were planted on the Cone tainer (3.81-cm diam. and

20.955-cm high; 1.5" diameter and 8.25" high), Stuewe and Sons, Inc., Tangent OR). ILeVO nematicide treated seeds were used as commercial check and non-treated seeds were used as a negative control. Cones were placed in the 2-gallon bucket filled with sand. There were two cones per treatment and all the treatments were fitted in a bucket, each bucket was considered as a block. Buckets were kept in the water bath where temperature was maintained 27° C throughout the experiment time with 16 hours of day light. Experimental design was two factorial completely randomized block design with six replications. Plants were watered every two days. 30 days after planting (DAP), one plant for each treatment was harvested from each bucket, while the remaining cones were left for another 30 days. Cones were taken out from the bucket and plants were excised above the soil line, plant height and fresh plant shoot weight of each plant was recorded. Cones were then soaked in the water for about 10 minutes for the ease of SCN cyst extraction. Each plant was uprooted, and the roots were sprayed with a strong stream of water to dislodge the SCN cysts. Cysts were collected in a 250- μ m sieve nested under a 710- μ m sieve. Root length and fresh root weight of each plant was measured and recorded. Cysts were counted under stereoscope and then later crushed by using motorized rubber stopper to release eggs. The eggs from the cysts were collected in 25 μ m sieve nested under 75 μ m sieve (Faghihi and Ferris 2000). Total number of eggs were counted under stereoscope and recorded. Experiment was repeated once.

3.2.6 Data analysis:

Data analysis was done using R studio version 3.5.3 (The R Foundation for Statistical Computing Platform). Total number of females and eggs per plant were standardized to per gram root mass before analysis. Analysis of variance (ANOVA) was used to determine

the effect of treatments on SCN egg hatching, reproduction, and soybean plant growth parameters. Least significant difference (LSD) test was used to conduct pair-wise mean comparison between treatments at $P \leq 0.05$ using R package “Agricolae”.

3.3 Results

Soybean cyst nematode egg hatching test:

Seed exudates: Based on the Bartlett’s test for homogeneity of variances (at $\alpha=0.05$), data from two experimental runs were combined and analysis of variance was conducted. On all the three time periods (day 3, 7, and 15), seed exudates collected from Saltro and ILeVO treated seeds significantly reduced the cumulative percentage SCN egg hatching in comparison to all the three controls (non-treated, ddH₂O, and 5 mM ZnSO₄) (Table 3.1). There was also significant difference between Saltro and ILeVO on cumulative percentage egg hatching across the days where, ILeVO significantly reduced the hatching than Saltro (Table 3.1).

Radicle exudates: Based on the Bartlett’s test for homogeneity of variances (at $\alpha=0.05$), data from two experimental runs were combined and analysis of variance was conducted. Effect of radicle exudates collected from Saltro nematicide treated seed on the cumulative percentage SCN egg hatching was not significantly different with hatching in ddH₂O on day 3 and day 7, but hatching was significantly different at day 15 (Table 3.2). There was significant reduction in SCN egg hatching due to the radicle exudates collected from ILeVO treated seed in comparison to all the controls (ddH₂O, non-treated, and ZnSO₄) across the days (day 3, 7, and 15) (Table 3.2). Radicle exudates from ILeVO treated seed

significantly reduced the hatching than radicle exudates collected from Saltro treated seed (Table 3.2).

Effect of Saltro on SCN cyst development: Based on the Bartlett's test for homogeneity of variances (at $\alpha=0.05$), data from two experimental runs were combined except for the two variables, total cyst per gram of root and total eggs per gram of root. These two variables were analyzed for each experimental run. In both runs, effect of treatments on the total cyst per gram of root and total eggs per gram of root was significant. There was significant interactive effect between seed treatment and days after planting (DAP), therefore data for 30 DAP and 60 DAP were analyzed separately.

30 DAP: Saltro nematicide seed treatment significantly reduced the cyst per gram of root and eggs per gram of root in comparison to the non-treated seed in the greenhouse conditions, whereas there was no significant difference between Saltro and ILeVO seed treatment on the both variables at 30 DAP (Table 3.3).

60 DAP: Saltro nematicide seed treatment significantly reduced the cyst per gram of root and eggs per gram of root in comparison to the non-treated seed in the greenhouse conditions. However, ILeVO seed treatment had significantly less cysts and eggs per gram of roots than the Saltro seed treatment (Table 3.3). Effect of nematicide seed treatment on the eggs per cyst was not significant (data not shown).

Effect of Saltro on soybean growth: Seed treatment significantly affected the plant height, fresh plant weight, and fresh root weight of the soybean plants in the greenhouse conditions (Table 3.4). Plant height, fresh plant weight and fresh root of the soybean plants at 60 DAP was significantly greater than at 30 DAP for all the treatments (Table 3.4). At 30 DAP and

60 DAP there was no significant effect of Saltro seed treatment on plant height in comparison to the non-treated control for both 30 and 60 DAP (Table 3.4). ILeVO seed treatment significantly reduced the plant height in comparison to the Saltro for both 30 and 60 DAP (Table 3.4). ILeVO seed treatment significantly reduced the plant height at 30 DAP in comparison to the non-treated control, but the effect was not significant at 60 DAP (Table 3.4).

At 30 DAP There was no significant effect of seed treatments on the fresh plant shoot weight in comparison to the control (Table 3.4). At 60 DAP there was no significant effect of Saltro seed treatment on the fresh plant shoot weight in comparison to the control, but ILeVO significantly increased the fresh plant shoot weight (Table 3.4). Saltro seed treatment did not affect the fresh root weight at 30 DAP, but significantly increased the fresh root weight at 60 DAP in comparison to the non-treated control (Table 3.4). ILeVO seed treatment significantly reduced the fresh root weight at 30 DAP, but significantly increased at 60 DAP in comparison to the non-treated control (Table 3.4).

3.4 Discussion:

Saltro seed treatment significantly reduced the soybean cyst nematode egg hatching and reproduction in lab and green experiments, respectively. Both seed and radicle exudates collected from Saltro nematicide treated seed greatly inhibited the SCN egg hatching in the lab experiments in comparison to water control. But the hatching was statistically higher in exudates collected from Saltro seed treatment than the ILeVO seed treatment. Seed and radicle exudates from Saltro treated seed inhibited SCN egg hatching by 55.4% and 37.5%, respectively. Whereas seed and radicle exudates collected from ILeVO treated seed

inhibited SCN egg hatching by 85.1% and 76.6%, respectively. In a similar study of accessing the effect of seed and radicle exudates collected from ILeVO treated seed Beeman and Tylka (2018) reported 98% reduction in SCN egg hatching due to seed exudates, whereas radicle exudates inhibited hatching by 48 % in one run had no effect in another. Effect of radicle exudate on the SCN hatching is less than the seed exudates in our experiment which might be due to low transfer of nematicide from the seed coat to the growing radicle tips. Faske and Starr (2007) have reported higher nematicidal effect of seed coat than emerging radicle on juvenile mortality associated with *M. incognita* when cotton seeds were treated with Abamectin.

Saltro nematicide seed treatment greatly reduced the total cysts per gram of root mass as well as total eggs per gram of root mass. We studied the effect of seed treatment for two different time intervals, 30 days after planting (DAP) and 60 DAP and effect was compared with the commercial chemical nematicide ILeVO. Saltro seed treatment reduced the reproduction of SCN as effectively as ILeVO at 30 DAP, while the effect was not like ILeVO at 60 DAP. Average number of cysts per gram of root was significantly greater at 60 DAP than 30 DAP for both seed chemical treatments.

As per our knowledge this is the first experiment intended to test the efficacy of Saltro nematicide, so we do not have additional information to compare between the efficacy results of Saltro across different experiments. But in refence to our experiment in greenhouse, Saltro is effective in controlling SCN, compared to untreated experiment but not effective as ILeVO. The percentage reduction of total cysts per root system for Saltro seed treatment is 90.5% and 80.3% in 30 DAP and 60 DAP, respectively compared to non-treated control. In comparison, ILeVO reduced total cysts per root system by 92.7% and

88.1% at 30 DAP and 60 DAP, respectively. Similar experiments have been conducted at different places to evaluate the efficiency of ILeVO on SCN. The percentage reduction of cysts per root system using same soybean cultivar (Williams 82) was found to be 35% at 30DAP in a greenhouse experiment conducted in IOWA (Beeman and Tylka 2018). Similarly, an experiment with seed treatment combining ILeVO with other seed treatments reported less SCN females per gram of root than with seed treatments excluding ILeVO. However, the effect of ILeVO on SCN reproduction was not (Zaworski 2014). Saltro seed treatment did not affect the plant growth parameters at 30 DAP but at 60 DAP, it increased the fresh plant weight and fresh root weight. ILeVO had negative effect on plant height and fresh root weight at 30 DAP. Similar findings of negative effect of ILeVO seed treatment on soybean plant at early stage (30 DAP) had been reported in various experiments (Beeman and Tylka 2018; Wise et al. 2015). On the contrary, ILeVO seed treatment increased the fresh plant weight and fresh root weight at 60 DAP. This shows that the negative effect of ILeVO seed treatment on soybean plants seen at early stage of plant growth are less damaging and plants recover from the injury at later stages (60DAP). Similar findings of later stage recovery of plants treated with ILeVO seed treatment has been discussed by Wise et al. (2015).

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Table 3.1. Effect of seed exudates collected from the soybean seeds treated with Saltro or Ilevo nematicides on cumulative percentage soybean cyst nematode egg hatching at 3, 7, and 15 days after treatments

Treatments	Cumulative egg hatch (%)		
	Day 3	Day 7	Day 15
Saltro	8.17 b	20.17 c	24 c
Ilevo	2.5 c	7.17 d	8 d
Nontreated	15.67 a	38.67 b	57.17 b
ddH ₂ O	13.17 a	38.33 b	53.83 b
5 mM ZnSO ₄	16 a	45 a	64.67 a

Each value is mean of 12 replications over two experimental runs. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 3.2. Effect of radicle exudates collected from the soybean seeds treated with Saltro or Ilevo nematicides on cumulative percentage soybean cyst nematode egg hatching at 3, 7, and 15 days after treatments

Treatments	Cumulative egg hatch (%)		
	Day 3	Day 7	Dy 15
Saltro	8.7 c	27.8 d	34.7 c
Ilevo	4 d	9 e	11 d
Nontreated	14.3 b	43.3 b	64.7 a
ddH ₂ O	11.3 c	34.2 c	55.5 b
ZnSO ₄	17.8 a	50.7 a	68.3 a

Each value is mean of 12 replications over two experimental runs. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 3.3. Effect of Saltro and ILeVO seed treatments on the reproduction of *Heterodera glycines* on soybean plants at 30 and 60 days after planting using field soil (2,000 eggs/100 cc soil) under greenhouse conditions

Treatments	Days after planting	Cyst per gram of root	Eggs per gram of root
Saltro		2.9 b	8.65 b
ILeVO	30	2.71 b	8.45 b
Nontreated		5.14 a	10.9 a
Saltro		4 b	9.74 b
ILeVO	60	3.42 c	9.16 c
Nontreated		5.77 a	11.51 a

Each value is mean of 12 replications over two experimental runs. Value for the cyst per gram of root and eggs per gram root were log transformed. Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test

Table 3.4. Effect of Saltro and ILeVO nematicide seed treatments on the growth of soybean at 30 and 60 days after planting under greenhouse conditions

Treatments	Plant height	Fresh plant weight	Fresh root weight
Saltro 30 DAP	16.05 c	1.86 c	1.58 cd
ILeVO 30 DAP	14.83 d	1.97 c	1.39 d
Nontreated 30 DAP	16.08 c	2.06 c	1.69 c
Saltro 60 DAP	22.13 a	3.46 b	2.60 a
ILeVO 60 DAP	21.16 b	4.0 a	2.63 a
Nontreated 60 DAP	21.7 ab	3.41 b	2.28 b

Each value is mean of 6 replications over two experimental runs. Plant height was measured in centimeter while weight was measured in gram

Means followed by the same letter within each column are not significantly different ($P < 0.05$) as indicated by Fisher's least significant difference (LSD) test.

Chapter 4

4 General Conclusions and Recommendations

The soybean cyst nematode remains the most important soybean pathogen whenever soybean is grown in the U.S. Determining alternative methods of SCN control is crucial given that host resistance has been breaking down due to limited resistance genes deployed and also due to limited choices of nematicides.

In this thesis, we report two different experiments. In first experiment, we tested the nematicidal activity of three weed species namely wormwood, ragweed, and leafy spurge, using extracts from dried plant materials in lab and green house condition. We found the varying degree of effects. Dried flower extract of Ragweed was most effective in inhibition of egg hatching, caused highest J2 mortality and greatly reduced SCN reproduction. In greenhouse condition, this plant extract treatment was as effective as chemical nematicide ILeVO to inhibit SCN reproduction.

However, we do not have any idea about which specific chemical in Ragweed flower that is responsible for its nematicidal property. Chemical analysis using High-performance liquid chromatography (HPLC) and testing potential chemicals individually will provide more insight in future. This information will be useful for formulating plant based nematicide. Also, individual chemical analysis will help to better explain the reason of stunted growth seen in soybean plants due to use of plant extracts. It is also needed to determine whether the effect in plant growth is limited to early stages or continues throughout the plant growth period.

In second experiment, we evaluated the efficiency of a new chemical seed treatment nematicide Saltro, comparing it with another common nematicide ILeVO. Saltro was not as effective as ILeVO, when assessed for its ability to inhibit SCN egg in the laboratory conditions. Saltro reduces the soybean cyst nematode reproduction as effectively as ILeVO in the early stage (30 days after planting) but the efficacy decreases at the later stage (60 days after planting) than the ILeVO. Additional experiments in field using Saltro needs to be done before concluding about the efficacy of this nematicide. Outcomes in the field cannot be certainly predicted to be similar as in the greenhouse experiments. This findings will help farmers to decide upon choosing a nematicide suitable for their condition.