

Effect of Giving Crab Shells as a Source of chitin on the Yellow Cyst Nematode (*Globodera rostochiensis* W) Population on Potato (*Solanum tuberosum* L.)

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ABSTRACT

This study aims to determine the effect of several concentrations of chitin on the NSK population and to determine the microbes (fungi) that act as chitinolytic microbes. The hypotheses put forward are (1) the higher the concentration of chitin, the lower the NSK population, (2) there are microbes (fungi) that act as chitinolytic microbes. to the soil at high concentrations. The best treatment capable of suppressing the yellow cyst nematode population and increasing the fresh weight of tubers was 0.5% chitin concentration. The results of isolation of soil microbes (fungi) before planting found 5 fungi that are chitinolic and capable of degrading chitin in cysts, namely *Trichoderma* sp., *Paecilomyces* sp., *Aspergillus* sp., *Verticillium* sp. and *Penicillium* sp., while the observation of 100 hst showed an increase in the fungus *Trichoderma* sp. from one species to four species. Potato (*Solanum tuberosum* L.) is an important horticultural commodity in Indonesia which is currently an alternative food ingredient, as a source of protein-rich carbohydrates to support food diversification programs (Rukmana, 1997). One of the important pests that are troubling farmers potatoes, especially in Malang district, East Java, is the Yellow Cyst Nematode (NSK). According to a report from the Ministry of Agriculture, in Indonesia in 2003 the area of infected plants was estimated at 25% of the total area of 800 hectares of potato plants. Control has been carried out well biologically and chemically. Alternatives for biological control include using materials that are unfavorable for the nematode environment, namely through the use of chitin.

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1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important horticultural commodity in Indonesia which is currently an alternative food ingredient and as a source of protein-rich carbohydrates to support food diversification programs. There are 14 known species in the genus *Globodera*, each with a specific host. There are two well-known species on potato plants, namely: *Globodera rostochiensis* known as the Yellow Cyst Nematode (NSK, Golden Cyst Nematode) and *Globodera pallida* (white yellow cyst nematode).

In Indonesia, the need for potato consumption is expected to continue to increase. The increased demand for potatoes was caused by the increasingly widespread utilization of potato production for various food ingredients, both as vegetables and snacks.

One of the risk factors in potato farming since in the field until it is stored is the presence of Plant Destruction Organisms (OPT). One of the important pests that is worrying potato farmers at the moment, especially in Malang Regency (East Java) is the Yellow Cyst Nematode (NSK), which is known abroad as the "golden cyst nematode" caused by *Globodera rostochiensis*. This type of nematode is a very dangerous nematode for potato plants, so the whole world is wary of it.

Control of Yellow Cyst Nematodes, among others, is regulated through laws by limiting seed production on affected land, using fumigant and non-fumigant nematicides, planting resistant varieties for 2 years and plants other than the Solanaceae family for 3 years (Ockey and Thomson, 2000). In addition, control can also be carried out mainly by utilizing biological agents, both fungi and bacteria, as well as adding materials that are unfavorable to the nematode environment (Hallmann, Rodriguez-kabana, and Kloepper, 1999). One alternative for nematode control is through soil improvement and the use of chitin. Adding chitin to the soil will stimulate the potential of chitinolytic microbes (microbes that are able to use chitin as a source of nutrition).

An increase in the chitinolytic microbial population will suppress the nematode population, because chitin is one of the main components of the nematode body wall (Sikora, Hagan, Gazaway, and Kemble, 2000), and chitin is mainly found in the skin or egg shells of nematodes (Doubrava and Blak, 1999). The results of the study using crab shell waste compost were able to suppress nematode populations. Applying crab shell waste compost of 10-20% (compost weight: soil weight) was able to suppress gall formation and egg mass production of *Meloidogyne javanica* in tomato plants. Meanwhile, 0.05% raw crab shells were more effective in suppressing root gall formation than using 20% crab shell waste compost (Peet, 2001).

When potatoes are planted, root exudate stimulates 60 to 80% of second-stage juveniles to emerge from the ova in the cyst (Stevenson et al., 2001). Infective second-stage larvae penetrate directly into young primary roots or the meristem tips of secondary roots. Furthermore, it enters the cortex intracellularly and causes damage and cell death. The larvae often pass through the cortex and insert their stylets into the cells of the endodermis or pericycle. During two days of penetration, the larvae rest and feed on cortex cells and stele tissue, causing cell swelling. The swollen cell groups are called syncytia, which are surrounded by a single layer of hyperplastic cells (Anonymous, 2004).

The nematode persists, feeds on swollen cells and molts twice then develops into the third and fourth juvenile stages. After the last molt, male worm-shaped nematodes emerge from the roots. Meanwhile, female nematodes grow and break the roots, so that their bodies protrude and only the head and neck are attached to the roots. They release a substance that attracts male nematodes, who then mate with them again. The embryo develops inside the egg to become a second juvenile, the egg remains inside the female. The cuticle of female *Globodera rostochiensis* is initially white and then becomes yellowish cream before turning golden and finally brown in the form of cysts (Stevenson et al., 2001).

2. METHOD

2.1 Types of research

The research method used in this study is the experimental method. The experimental method is a form of observation under artificial conditions, where these conditions are created and regulated by the researcher. That is, basically conducting an experiment to see the results, and the results of the experiment will confirm how the causal position is between the variables being investigated

2.2 Research variable.

The variables observed were the number of cysts and the fresh weight of tubers. Observations were made destructively at 50 days after planting and 100 days after planting. Variable number of cysts in the soil, observations were made by taking 0.5 kg of soil in the area around the roots, then drying it in air for 1 day. Data on the number of cysts was obtained from the extraction of cysts in 100 grams of dry soil.

2.3 Research design

The study was carried out in a completely randomized design (CRD) with 5 treatments and 4 replications. The treatments tested were: 0 grams of crab shell powder/pot (0% chitin) = P1; 125 grams of crab/pot shell powder (chitin 0.5%) = P2; 250 grams of crab/pot shell powder (chitin 1%) =

P3; 375 grams of crab/pot shell powder (chitin 1.5%) = P4; 500 grams of crab/pot shell powder (chitin 2%) = P5

2.4 Sampling location

This research was carried out with samples of Yellow Sista Nematode Cysts from infested potato fields in Sumber Brantas, Tulung Rejo village, Bumi Aji sub-district, Batu city and Granola potato tubers.

2.5 Time and Place of Research.

The research was conducted in a screen house in Pesanggrahan Village, Batu City and Plant Disease Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Brawijaya University from August 2006 to November 2007.

2.6 Tools and materials

The tools used in this study were plastic containers, Petri dishes, spatulas, spray bottles, fine brushes, microscopes, 50 and 60 mesh filters, 600 ml measuring cups, 10 ml measuring cups, plastic trays, cutter, scissors, scales, small plastic and large, 10 kg polybag, gauze, trowel or hoe, hand-counter, mortar, stove, sterile media bottles, test tubes, loops, pipettes, Bunsen, label paper, glass objects.

Materials used in this study include: Sista Yellow Sista Nematodes originate from infested potato fields in Sumber Brantas, Tulung Rejo village, Bumi Aji sub-district, Batu city; Soil as a planting medium. The soil used is free of Yellow Cyst Nematode infestation and has an andosol soil type. Before being used, the chitinolytic fungi and the nutrient content in the soil were analyzed first; Potato tubers of the second generation Granola variety which are ready for planting are one month old. Selected bulbs that have the same size (approximately 5 cm in diameter); NPK compound fertilizer; Chitin which comes from crab shell material in powder form; Insecticides and Fungicides; Chitin-agar medium. Media was prepared with ingredients (10 gram pure chitin, 1000 ml aquades, 40 gram salt, 50 ml HCL). Ingredients 1, 2 and 3 are boiled until boiling and removed from the stove, after which the HCL is slowly poured into the media using a pipette. The mixture of these ingredients is put in a media bottle and autoclaved. The media was then mixed with 20% water-agar (20 gram agar and 1000 ml distilled water) as a medium for growing chitinolytic fungi (Logan et al., 2005).

2.7 Research procedure

The study began with the crab shells being dried in the sun, then pounded until smooth using a mortar and sifted through a 20 mesh size sieve (Hallmann et al., 1999). After that it is put in plastic and stored until application time. The percentage of chitin content in the material was obtained from laboratory analysis results of 32% and preparation of planting media and potato planting, cyst inoculation and plant maintenance as well as testing for chitinolytic microbes (fungi).

2.8 Data analysis.

The data obtained were analyzed using the F test at the 5% level, then if there was a significant difference it was continued with the BNT level test 5%.

3. RESULTS AND DISCUSSION

3.1 Research result

3.1.1 Number of cysts per potato plant

The results of the analysis of variance on the average number of cysts per potato plant indicated that the treatment with crab shell powder as a source of chitin had a significant effect on the Yellow Cyst Nematode population.

Table 1. Average number of cysts per 100 g of soil in each treatment

treatment	The number of cysts on observation			
	50 hst		100 hst	
	Original data	Transformation (*)	Original data	Transformation (*)
Chitin 0%	3.25	0.51a	160.5	2.2 a
chitin 0.5%	2.25	0.35a	45.5	1.65 b
chitin 1%	1.5	0.15b	24.25	1.38c
chitin 1.5%	1.25	0.08b	18	18 1.25 d
chitin 2%	1	0.b	10	0.99e

Note: Numbers accompanied by the same letter in the same column, not significantly different in the 5% BNT test; hst = days after planting. (*) The mean has been transformed to log 10.

Observations on the average number of cysts at 100 hst showed that the Yellow Cyst Nematode population decreased in all treatments with the addition of chitin compared to without the addition of chitin. The 0% to 2% chitin treatment was significantly different among all the treatments tested. This shows that the crab shell powder tested was able to suppress the development of the Yellow Cyst Nematode population. The number of cysts in the 0% chitin concentration treatment did not increase from the initial population (300 cysts), presumably because the ability of Yellow Cyst Nematodes taken from the field decreased, due to different altitudes, the attack of microorganisms or the application of pesticides in the field by farmers.

The number of cysts at 50 dap was lower than that at 100 dap. This indicates an increase in the Yellow Cyst Nematode population over a certain period of time (50 hst). The increase occurred significantly. Observation of the cyst population at 100 hst showed that there was a decrease in the number of cysts in the treatment with the addition of chitin when compared to without the addition of chitin. The best treatment when compared to the control was at 2% chitin concentration. The higher the concentration given, the number of cysts in the soil continues to decrease.

3.1.2 Wet Weight of Potato Tubers.

The results of the analysis of variance of the average wet weight of potato tubers at 50 and 100 days after planting (DAP) showed that the treatment with chitin had a significant effect on the fresh weight of the tubers.

Table 2. Average fresh weight of potato tubers in each treatment

Treatment	Fresh Weight of Tubers on Observation	
	50 hst	100 hst
chitin 0%	38,825	47.1 bc
chitin 0.5%	42.35	49.2c
chitin 1%	2:65b 35	325 bc
chitin 1.5%	12,575b	12,575
chitin 2%	4.95a	14.2 a

Note: Numbers accompanied by the same letter in the same column are not different real on the 5% BNT test; DAP = days after planting.

At 50 hst observation, there was a decrease in fresh weight of tubers treated with chitin compared to without chitin, except at 0.5% chitin concentration. Treatment of 0% chitin was not significantly different from 0.5% chitin. Giving chitin concentration of 1% was not significantly different from 1.5% chitin. Meanwhile, 2% chitin was significantly different from all the treatments tested. The fresh weight of tubers at 100 dap increased compared to 50 dap, although there was no significant increase during the period (50 dap). The average wet weight of potato tubers aged 100 dap at all treatments increased from initial weight (50 hst). At 0% and 0.5% chitin treatment, the increase in fresh weight of tubers was lower when compared to 1% to 2% chitin. Administration of chitin with concentrations exceeding 0,

3.2 Discussion.

Giving crab shell powder as a source of chitin to the soil can suppress the development of the Yellow Cyst Nematode (NSK) population. Population decline is shown by the reduced number of cysts in the soil when compared to the control. Test results for several chitin concentrations (0% to 2% per soil weight) on potato plants aged 50 and 100 DAP on the NSK population showed that the higher the concentration of chitin given, the number of cysts in the soil decreased. This proves that the greater the concentration of chitin given, the NSK population will decrease. The addition of 125 g of crab shell powder or chitin equivalent at a concentration level of 0.5% to the soil can reduce the NSK population to 45.5 cysts per 100 grams of soil, approximately 71.65% when compared to controls. Chitin concentration of 1% was able to reduce the NSK population by up to 24.3 cysts or 84.86% when compared to controls. While the number of cysts in the soil at a concentration of 1.5% to 2% is less when compared to the 0.5% and 1% chitin treatment. These results are in accordance with the research of Tarno et al. (2000), which showed that giving 149.1 g of shrimp shell powder or chitin equivalent at a concentration of 1% was able to suppress NSK populations up to 39.25 cysts per 100 g of soil.

The mechanism for changing conditions in the soil that does not support the development of the Yellow Cyst Nematode is not directly carried out by chitin, but is related to increased chitinolytic microflora and chitinase activity which can increase parasitism in cysts as stated by Spiegel et al., 1987 (in Brown et al., 1995). This is also as revealed by Mian et al., 1982 (in Bell et al., 2000), that the mechanism of nematode control by chitin is related to increased activity of microbial chitinase which damages the chitin in the eggshell. Meanwhile, Guerenam (2006), said that the addition of

materials containing chitin (crab shells), not only significantly reduced the number of parasitic nematodes, but also effectively increased the population of chitinolytic fungi that attack nematode eggs and other structures of nematodes (which contain chitin).

4. CONCLUSION

Application of 125g of crab shell powder/pot or the equivalent of chitin at a concentration level of 0.5% (chitin : soil weight) into the soil was able to suppress the Cyst Nematode population and increase tuber yield and the higher the concentration of chitin given (0.5% up to 2%), the Yellow Cyst Nematode population is decreasing and from the isolation of soil fungi and pathogenicity tests on cysts, chitinolytic fungi are obtained that are able to degrade cysts, namely *Trichoderma* sp., *Paecilomyces* sp., *Aspergillus* sp., *Verticillium* sp. And *Penicillium* sp.

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Tests were carried out in the field to obtain more accurate information and analysis of diversity and microbial tests and further tests were carried out with the addition of hemicellulose to reduce the phytotoxic effect of chitin on plants. as a whole (fungi, bacteria and actinomycetes) against cysts to find out which organisms are most effective and have the potential to control Yellow Cyst Nematodes. as well as testing with a range of chitin concentrations below 0.5% and the effectiveness of chitinolytic fungi against NSK on potato plants in pots.

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