THE COUNTRYSIDE DEVELOPMENT RESEARCH JOURNAL

Vol. 8 Issue 1 | 2020

Application of Vesicular Arbuscular Mycorrhiza (VAM) and Effective Microorganism (EM) against Root Knot Nematode (*Meloidogyne incognita* Chit.) in Tomato (*Lycopersicon esculentin* L).

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Article Information

History:
Received: 24JUL20
Final Revision: 30AUG20
Accepted: 02SEPT20

Keywords:
Effective microorganism
Nematode
Tomato
Vesicular arbuscular mycorrhiza

Abstract: *The study was conducted to evaluate the efficacy of Arbuscular Mycorrhizal (AM) fungi and Effective Microorganism as bio-protectant to root-knot nematode (Meloidogyne incognita Chit.) and to evaluate its effects on the agronomic characteristics and yield performance of tomato (Lycopersicum esculentum L.). The experiment was laid out in RCBD with eight treatments, replicated four times with nine plants per treatment. The treatments evaluated were as follows: (T1) Complete fertilizer (14-14-14); (T2) Complete fertilizer (14-14-14) + Urea (45-0-0); (T3) VAM at 540 g/plot (T4) EM at 1,350 g/plot; (T5) VAM 540 g/plot + EM at 1.30 g/plot; (T6) VAM at 540 g/plot + 14-14-14 at 72.39 g/plot; (T7) EM at 1,350 g/plot + 14-14-14 at 72.39 g/plot and (T8) VAM at 540 g/plot + EM at 72.3 g/plot + 14-14-14 at 72.3 g/plot. Effectiveness of such treatment was based on root gall counts, number of adult nematodes, weight of the roots with root galls, plant height, and yield performance of the tomato. Compared to other treatments, a combination of VAM & EM & 14-14-14 (T8) had the lowest number of nematodes, lowest weight of root galls with nematodes, and gained the lowest weight of fresh roots with galls. This combination effectively controlled nematodes population and increased yield of tomato (var. Marimar) by 10% under field condition compared to all treatments.*

I. INTRODUCTION

Tomato (Lycopersicon esculentum, Miller) locally known as “kamatis” is one of the most popular vegetables in the tropic and considered as the most important vegetable in Asia. It is a source of income for Filipino farmers and small gardeners since it is commonly grown in any month of the year, preferably from May to September (Cubero, 2017). However, tomato production is confronted with several problems because of many diseases and pests.

One of the unnoticed pests that have been infecting many crops nowadays is the Root-Knot Nematodes (RKN). This pathogen is obligate plant-parasitic that are widely distributed worldwide, RKN consist of 98 species which parasitize almost every vascular plant. The vernacular name comes from the word galls (root-knot) induced by *Meloidogyne spp.* in the roots of their host (Jones et al., 2013). When *M. incognita* attack the roots of its host plants, it sets up a feeding location where it deforms the normal root cells and establishes giant cells. The roots become gnarled or nodulated, forming galls, hence the term “root-knot” nematode. These galls measure anything from 1 to 20 mm across and often coalesce, causing considerable distortion (David, 2012). Over the year, plant resistance and nematicides have been used against nematode attacks but have led for many pathogens to breakdown resistance due to severe selection and too much use of chemicals (Aubertot et al., 2006).

Many bio-protectants are being used to control Root-Knot Nematodes (RKN) and one of these is the use of Effective Microorganisms (EM) which is capable to influence decomposing organic matter such that it reverts into a life-promoting process. Effective Microorganisms are also called EM technology by the proponents and are used to maintain sustainable practices such as farming and sustainable living and to support human health and hygiene. One of the principal activities of EM is to increase the biodiversity of soil flora resulting to increase crop yield (Olle & Williams, 2013).

It was also hypothesized that organic compost may increase soil biological fertility more effectively than mineral fertilizers. Effective microorganisms could improve the effects of traditional compost. Condor et al., (2007) state that effective microorganism mixed with naturally occurring microorganisms like photosynthetic bacteria will synthesize amino acids, nucleic acids, bio-active substances, and sugars, using substances from the root secretions, organic matter (carbon), sunlight, and geothermal heat from the soil as sources of energy. The EM interacts with the soil-plant ecosystem to control plant pathogens and at the same time maintain the microbial and ecological balance of the soil, resulting also to increase photosynthetic activity. So far, few investigations have analyzed the effects of the effective microorganisms on soil fauna such as nematodes.

On the other hand, the Vesicular-Arbuscular Mycorrhiza (VAM) is recognized as bio fertilizer due to its manifold advantages provided to the host plants by increasing nutrients and water uptake (Bohra et al., 2007). The use of VAM and the penetration of root-knot nematodes *Meloidogyne incognita* in tomato was studied. The number of giant cells formed in mycorrhizal plants was significantly low. The Arbuscular Mycorrhizal Fungi (AMF) promote rapid increase in plant growth development.

In nursery setup, VAM protect and improve growth and protect the plants from soil borne pathogens including nematodes (Anjos et al., 2010). The control of root-knot nematodes using VAM fungi in combination with EM is not well documented and little experimental evidence is available under local conditions. This study aims to compare the effectiveness of EM and VAM against root-knot nematode of tomato under field conditions, specifically to evaluate the effect of VAM and EM against root-knot nematode in terms of agronomic characteristics, symptoms, and yield performance of tomato.

II. METHODOLOGY

Research Design

The experimental setup was conducted at the Experimental Station of the College of Agriculture, Nueva Vizcaya State University-Bayombong Campus. The experiment was laid out in a Randomized Complete Block Design (RCBD) with eight treatments and four replications. Eight plots were assigned to each replicate or block. The distance between plots is 0.5m x 1m in a row with an area of 500m^2.

The experimental treatments are designed as follows:

Treatment	Treatment Description
T_1	Control
T_2	14-14-14 at 144.6 g/plot
T_3	VAM (540 g/plot)
T_4	EM (1,350 g/plot)
T_5	VAM (540 g) + EM (1,350 g)
T_6	VAM (540 g) + 14-14-14 (72.3 g)
T_7	EM (1,350 g) + 14-14-14 (72.3 g)
T_8	VAM (540 g) + EM (1,350 g) + 14-14-14 (72.3 g)

Research Samples

Acquisition and Variety Used

Seeds of Marimar F1 used in the study was purchased from a reliable agricultural supply at Bayombong, Nueva Vizcaya. Seeds of Marimar F1 were sown singly in a seedling tray to produce healthy and uniform seedlings and to prevent transplanting shock. The tray used has 101 cells, each of which was filled with one-part compost, processed animal waste and 1-part carbonized rice hull.

Preparation of Nematode Culture and Inoculation

Infected tomato plants from the field with typical galls or knots were collected. Roots were gently washed with water, and under a dissecting microscope single mass from cut galled roots were picked out. Egg masses were incubated for 2-4 days at room temperature to allow hatching of second-stage juveniles (J2). The hatched J2 were inoculated into 20-day old tomato plants *(Solanum Lycopersicum L.)* in the experimental field. These inoculated plants were maintained till 3, 6, 9, 12 and 15 weeks for destructive sampling.

Cultural Management of Host Plants

Proper care and management were done throughout the whole duration of the experiment from Seedling, Land Preparation, transplanting of the seedlings, Cultivation, Weeding, Trellising, Irrigation, and Harvesting. Strict daily monitoring was done also to observe different occurring insects and diseases. Hand-picking was implemented on insect pests.

Data Collection Method

The data gathered in this study were categorized into agronomic characteristics of the test plant, yield, the number of root galls formed by the *M. incognita,* the number of juvenile and adult nematodes, and were recorded after 3, 6, 9,

12 weeks after transplanting (destructive sampling) till harvest. The fresh weight of roots

ISSN 2449-4577 (online) 2408-283X (print)

with galls was recorded after harvest. The weight of root galls (g) was obtained by separating the galls from the roots before it was weighed. This was done also after harvest. The plant height (cm) was measured from the base of the plant to the tip of the highest leaf of the plant after harvest. Lastly, for the yield (g), fruits were gathered as soon as it matured from the 4th week to the 15th week.

Other symptoms/abnormalities expressed by the plants were observed from transplanting to harvesting due to other pests.

Data Analysis

The data were statistically analyzed using the two-way classification of the Analysis of Variance (ANOVA) for Randomized Completely Block Design. Significant differences among treatment means were compared using Duncan's Multiple Range Test (DMRT).

Ethical Consideration

The study did not involve any human and animal samples. Plants used in the study is solely for experimental purpose only. There is no conflict of interest, and received no funding from any funding agencies.

III. RESULTS AND DISCUSSION

Table 1 presents the summary data on fresh weight of roots with galls, the weight of root galls, number of adult nematodes, yield and plant height of tomato after harvest as affected by the application of inorganic fertilizer (14-14-14), Vesicular Arbuscular Mycorrhiza (VAM) and Effective Microorganism (EM).

Weight of Fresh Roots with Galls after Harvest

Table 1

Summary of Data

Treatment	Weight of Fresh Roots with Galls (g)	Weight of Root Galls (g)	Number of Adult Nematodes	Fresh Yield of Tomato (kg)	Plant Height (cm)
T_{1}- Control	92.50^{a}	6.97^{a}	228.50^{bc}	9.65^{c}	109.00
T_{2}- 14-14-14	21.00^{f}	2.32^{c}	38.75^{d}	18.58^{a}	105.75
T_{3}- VAM	27.75^{e}	4.25^{b}	306.00^{b}	8.69^{c}	95.50
T_{4}- EM	58.75^{b}	1.50^{d}	108.50^{cd}	11.80^{bc}	104.88
T_{5}- VAM+EM	30.00^{d}	11.25^{a}	573.75^{a}	10.04^{c}	106.25
T_{6}- VAM+14-14-14	32.50^{c}	6.50^{a}	226.00^{bc}	10.77^{c}	104.75
T_{7}- EM+14-14-14	17.50^{g}	6.50^{a}	88.00^{cd}	19.22^{a}	119.50
T_{8}-VAM+EM+14-14-14	13.00^{h}	0.75^{e}	17.00^{d}	18.59^{a}	108.00

Note: In a column, all means with the same superscripts are not significantly different at 5% level of significance by DMRT.

Treatment 1 (Control) and T_4 (EM) obtained the highest fresh roots with galls with means of 92.50 and 58.75 followed by T_6, T_5, T_3, T_2, and T_7 with means of 32.50, 30.00, 27.75, 21.00, and 17.50, respectively. Treatment 8 obtained the lowest mean of 13.00.

Further analysis showed that T_4 (EM) significantly displayed the highest weight of roots with galls with a mean of 58.75. On the other hand, Treatment 8 (VAM + EM + 14-14-14) significantly showed the lowest weight of root with galls with a mean of 13.00 and significantly different when compared with the other treatment. Consider that T1 obtained the highest weight of Fresh Root with galls because there was no intervention or any control applied on the test plant, while the EM was in favor of the occurrence of Root Knot Nematodes (RKN).

This is in accordance also to the study of Hu and Qi (2013) on wheat where they compared the effectiveness of Effective Microorganism and traditional compost. Result revealed that the total number of nematodes population was higher when applied with Effective Microorganism compared to traditional compost plot and free leaving nematodes are more abundant for Effective Microorganism than on the traditional compost. The increase number of nematodes in the fresh roots can be directly linked to the greater abundance of food associated with the EM and compost or naturally in the soil (Villenave et al., 2003).

Weighted of Roots Galls

The average weight of galls after harvest is shown in Table 1. Data ranged from 0.75g to 11.50g. Results have shown that T_8 (VAM+EM +14-14-14) had the lowest weight followed by T_4 (EM) T_2 (14-14-14) and T_3 (VAM). On the other hand, T_1 (Control), T_5 (VAM+EM), T_6 (VAM+14-14-14), and T_7 (EM+14-14-14) obtained higher gall weight. Comparison among treatment means revealed that T_1, T_5, T_6, and T_7 were comparably higher in terms of gall weight but significantly different from T_2, T_3, T_4, and T_8.

Further analysis showed that T_8 (VAM + EM + 14-14-14) obtained the lowest mean in terms of gall weight after harvest (Table 1). The result could be attributed to the combined effects of the treatments to control the nematode population causing lesser gall formation in the root system.

The result aligns with the study of Begum et al. (2019) emphasized that VAM and 14-14-14 treated plants showed lower root galls in Marimar variety. Given that there were a lot of negative effects of chemical fertilizer on the soil ecosystem because it decreases the soil biodiversity, on the study, it significantly attributed on the control of nematodes. Same result was obtained in the study of Hu and Qi (2013) which suppressed nematode population on both parasitic nematodes and free living nematodes.

Number of Adult Nematodes

This was taken to determine the duration of the efficacy of all the treatments as observed in Table 1. Treatment 5 obtained the highest number of adult nematodes with a mean of 573.75 followed by T_1, T_3, and T_6 with means of 228.50, 306.00 and 226, respectively. On the other hand, plants in T_8, T_2, T_4, and T_7 have lower means of 17.00, 38.75, 88.00, and 108.50, respectively. Statistical analysis showed that T_8 (VAM + EM + 14-14-14), T_2 (Control), T_4 (EM) and T_7 (EM + 14-14-14) were comparable in controlling the population of adult nematodes followed by T_1 (Control), T_4 (EM), T_5 and T_6.

On the other hand, T_2 and T_8 comparably obtained the lowest means showing the combined effects of VAM+EM+14-14-14 on the control of the nematode population (Table 1). The abundance number of Adult RKN produced by Treatment 5 correlated to the study of Ekschmitt et al. (2001), that application of Effective Microorganism contributes to greater biodiversity in the soil habitat compared to those applied with NPK fertilizers or in combination to VAM. Moreover, Linderma and Davis (2003) reported that Vesicular Arbuscular Mycorrhiza (VAM) inhibits the growth of soil pathogens, protects the roots from pathogen invasion, and improves the resistance of plants against soil pathogens, but in the study when VAM was combined with EM, population of nematodes increased, thus, application of EM may affect the performance of VAM to control nematodes population.

Fresh Yield of Tomato

Table 1 also shows the fresh yield of tomatoes taken after harvest. Treatment 7, T_2, and T_8 obtained the highest means with 19.22, 18.58, and 18.59, respectively, followed by T_4 with a mean of 11.80. Treatment 1, T_3, and T_5 obtained the lowest yield with means of 8.69, 10.04, and 10.77, respectively. Statistical analysis showed that T_7 (EM+14-15-14) T_2 (14-14-14) and T_8 (VAM+EM+14-14-14) were comparably higher in terms of fresh yield but significantly different from T_4 (EM). On the other hand, T_1 (Control), T_3 (VAM), T_5 (VAM + EM) and T_6 (VAM + 14-14-14) were comparable in terms of fresh yield but significantly different from the other treatments. The significant result could be attributed to combined effects of VAM, EM, and 14-14-14 on the nematode population and the nutritional value.

It is believed that mycorrhiza, as reported by Kadian and Aggarwal (2018), changes the attractiveness of roots to nematode infection and reduced survival by producing antibiotics and auxins which immobilize, kill, and inhibit cell membrane synthesis in developing eggs, juveniles, and adult nematodes. On the other hand, the Vesicular Arbuscular Mycorrhiza (VAM) is recognized as biofertilizer due to its manifold advantages provided to the host plants by increasing nutrient and water uptake (Bohra, 2007). Likewise, Hu and Qi (2013) hypothesized that organic compost may increase soil biological fertility more effectively than mineral fertilizer, while effective microorganisms (EM) also improve the effectiveness of traditional compost.

Plant Height

In terms of the pant height of the tomato which ranged from 95-120 cm taken after harvest, Treatment 7, T_1, and T_8 and T_5 obtained the highest mean in terms of height with means of 119.50, 109.00, 108.00, and 106.25 followed by T_2, T_4, T_6 and T_3 with means of 105.75, 104.88, 104.75 and 95.50, respectively. Statistical analysis revealed no significant differences among treatments. The mycorrhizal tomatoes did not show a significant effect with non-mycorrhizal tomatoes. This is also true for the other treatments.

The result could be attributed to the concentration of inocula that affects the plant's height. Kadian and Aggarwal (2018) reported that measurement of the height of plants and length of leaves during growth indicated that inocula containing a low number of spores stimulated the host less than more in concentrated inocula. Plants inoculated with a high number of spores

ISSN 2449-4577 (online) 2408-283X (print)

produced more on upper and retained lower leaves.

Other Observation

Blossom-end rot disease was observed from unripe and ripe fruits from the first to the last harvest. Curling, yellowing, and stunting were also observed in a few plants during the vegetative stage.

IV. CONCLUSION

Based on the study conducted, experiments related to nematodes give more insights and additional literature for the control of unnoticeable pathogens like Root Knot Nematodes (RKN). This also contributes knowledge about inputs that we have been introduced to suppress pest infestation. The use of combined bioprotectants like VAM, EM, and in combination with NPK can significantly reduce the RKN population in Tomato, as to reflect other studies that mycorrhiza inhibits the growth of soil pathogens and also protects the roots from the pathogen. It was also shown that the combination Mycorrhizal fungi + Effective Microorganism + Inorganic Fertilizer increased yield of tomato (var. Marimar) by 10% under field condition compared to other treatments.

The plants exposed to Mycorrhiza and Effective microorganisms (EM) had significantly reduced *the Meloidogyne* population and increased Tomato's yield under field conditions. Thus, the result of the study recommends that Mycorrhiza combined with EM and Inorganic fertilizer is favorable to recommend to farmers as it reduced health hazards and will not contaminate the environment, thus, conserving the natural enemies and it fits well with the organic crop production system especially in the countryside area of tropical countries.

ACKNOWLEDGMENT

The authors acknowledged the help of Dr. Jonar I. Yago, Prof. Ermelito Irabagon, and the College of Agriculture, Nueva Vizcaya State University, Bayombong Campus support extended on the analytical and technical aspects of the study.

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ISSN 2449-4577 (online) 2408-283X (print)