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EFFICACY OF CULTURAL METHODS IN THE CONTROL OF *RHIZOCTONIA SOLANI* STRAINS CAUSING TOMATO DAMPING OFF IN KENYA

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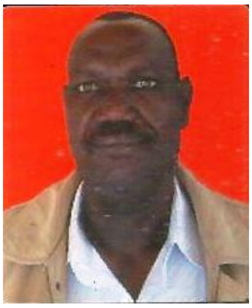

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ABSTRACT

Rhizoctonia damping off of tomato caused by *Rhizoctonia solani* can be a serious problem in most intensive production environments. Recent increase in smallholder vegetable production of 0.5-3.0 ha in Kenya has resulted in build up of the pathogen to above economic threshold levels. There is no effective chemical control or resistant varieties. Use of soil fumigants such as methyl bromide in the control of Rhizoctonia and other soil borne pathogens is not sustainable due to their high costs, and toxicity to man and environment. Cultural methods such as soil amendments, mode of planting and influencing soil moisture levels either alone or in combination with other methods are among the most likely substitutes to use of toxic fumigants for control of soil borne pathogens in agriculture. This study was undertaken to evaluate the effects of cow manure application, transplanting, planting on raised beds and varying watering interval on Rhizoctonia damping off of tomato as measured by percent seedling survival at 5-30 days after planting (DAP), disease severity at 30 DAP, percent crop stand at 60 DAP and fruit yield at maturity. The study was conducted in a field artificially inoculated with pathogenic strains of *Rhizoctonia solani* isolated from infected tomato plants and Rhizosphere soil sampled from the major production regions of Kenya. The efficacy of the various cultural strategies both singly and in combination on the Rhizoctonia damping off management was compared with the conventional disease control involving chemical fumigation with metham sodium and two chemical seed dressers (pencycuron, thiram, imidacloprid) and (captafol) as the standard. Cow manure application and shorter watering interval when used singly or in combination with other cultural methods produced lower disease control and yield. Transplanting, planting on raised beds and medium irrigation interval when used singly or in combination with other cultural strategies produced good disease control resulting in higher yield that compared favorably with the conventional disease control involving soil fumigation and seed dressing. The various cultural disease control methods documented in this study can be used alone and in integration with other compatible Rhizoctonia damping off of tomato control strategies. Furthermore the promising non-chemical strategies may form part of the urgently sought for alternative to use of hazardous fumigants in agriculture and since they are not specific, there is low risk of resistant development over time.

Key words*:* Cultural disease control, *Rhizoctonia solani*, tomato

INTRODUCTION

The soil borne plant pathogen *Thanatephorus cucumeris* (Frank) Donk [anamorph]: *Rhizoctonia solani* (Kun) is a basidiomycete that occurs worldwide and causes economically important diseases to a large variety of vegetable and field crops, turfgrasses, ornamentals, and fruit and forest trees; inflicting yield losses averaging up to 20% yearly in over 200 crops worldwide [1, 2, 3].

In Kenya, Rhizoctonia damping off is a serious disease of tomato and other vegetables such as kales, beans, okra, egg plant and flowers with up to 30% yield loss. Intensive vegetable production and lack of effective disease management strategies has resulted in buildup of the pathogen to above economic threshold levels in most production locations. Effective control of the disease is being hampered by lack of cost-effective and sustainable control methods. Most of the popular tomato varieties grown locally are susceptible and rotations are often ineffective due to the wide host range and lack of adequate land [4].

Although various non-fumigant and seed dressing fungicides are available in the market for the control of the disease, they are expensive, toxic and their effectiveness is questionable probably due to multiple genera and species of root rot pathogens that occur simultaneously, and biodegradation after continued use [5, 6]. Soil disinfestation is through drastic means as fumigation with general biocides such as methyl bromide or steaming [7, 8]. However, the recent decision by the United Nations to ban the use of methyl bromide in agriculture by 2015 due to its hazardous nature is a major setback in the control of *Rhizoctonia* and other soil borne diseases [9, 10].

This study intends to meet the challenge of research to define and implement a cost effective and sustainable non-chemical strategy for controlling Rhizoctonia damping off of tomato on smallholder vegetable farming in Kenya. The cultural strategies evaluated are effects of influencing soil organic matter content, mode of planting, and soil moisture levels through drainage and varying watering interval either singly or in combination.

MATERIALS AND METHODS

Experimental design and layout

This two-season open field experiment was conducted during the dry months of July-October 2004 (22-25 ^{0}C) and January-March 2005 (22-26 ^{0}C) at the University of Nairobi's Kabete farm to evaluate the effect of four cultural strategies (singly and in combination) commonly practiced by tomato farmers in Kenya on Rhizoctonia damping off of tomato. The soils at the site were deep loam to clay with pH of 7.5-8.5. The specific treatments were effect of planting on raised beds, effect of cow manure application, effect of varying watering interval, effect of transplanting, effect of raising beds + cow manure application, effect of cow manure application + varying watering interval, effect of raising beds + varying watering interval and effect of raising beds + varying watering interval + cow manure application. The cultural strategies that registered positive effect(s) on Rhizoctonia damping off control as assessed by percent seedling survival at 30 DAP, disease severity at 30 DAP, percent crop stand at flowering, and quality and quantity of yield at maturity were compared with chemical fumigation using a general biocide fumigant-trade name Basamid 800 Gr (metham sodium; methyl isothiocyanate) and two seed dressing chemicals-trade names Gaucho MT 390 FS (pencycuron 50 g/l, thiram 107 g/l, imidacloprid 233 g/l) and Captan 1200 EC (captafol; a carbendazim) as the standard check and uninfested as the control. Trials involved three *R. solani* susceptible commercial tomato varieties Caltana, Roma and Marglobe commonly grown in Kenya against a mixed inoculum of 56 pathogenic strains of *R. solani* extracted from infected tomato seedlings and rhizosphere soil sampled from major production regions of the country. The isolated *Rhizoctonia* was identified by morphological and cultural characteristics of pure isolates on acidified potato dextrose agar, the 56 strains differentiated by gene typing using microsatellite technique and their pathogenicity on the three tomato varieties confirmed under greenhouse experiment. The experimental design used in this study was a split plot in complete randomized block design with 4 replicates. Randomization of 2nd, 3rd and 4th replicates was done in the field during trial set up. Microplots measuring 2.7 M long and 2.1 M width were used as experimental unit and replicated four times to make an experimental block. Each treatment block was made up of sum of its experimental units and, therefore, the number of plants on each depended on the number of the constituent experimental blocks.

In the direct seeding used in all experiments except the transplanting, the experimental unit was made up of one row per variety having five planting hills each planted with five seeds which was replicated four times to make a treatment block. This was compared with transplanted seedlings in which 21 days old *Rhizoctonia* free seedlings planted at a rate of one seedling per hill made an experimental unit. Bed raising was done up to 10 cm using a forked hoe. Flat bed was the even planting field. Steam sterilized dry cow manure of C: N ratio 10:11 in a cattle shed (Boma) was applied by hands. The three rates evaluated were 0 cm^3/hill, 150 cm^3/ hill and 300 cm^3/hill. The four levels of watering regime evaluated were normal watering regime, watering daily, watering alternate days and watering after two days. Watering was done manually by use of a watering can to about 70% field capacity (normal watering) which was determined by use of a tensiometer. Normal watering refers to optimum application of water to avoid any negative effects of water stress on the crop. This translated to watering twice daily during the first week at 1000 cm^3/hill, once daily at 1000 cm^3/hill from establishment to fruiting and 2000 cm^3/hill till harvesting at ambient temperatures of 22-26 ^{0}C and loam/clay soil. A particular watering interval was maintained throughout the season.

Fumigation with Basamid was done by inoculating a prepared flat bed with *R. solani* at 1000 cm^3 of soil potato inoculum/M^2 (dispersed by hand on the surface) and then Basamid granular at the rate of 5 g/M^2 dispersed on top and mixed with the soil using a forked hoe. The bed was then sprinkler irrigated up to 70% field capacity and covered with a transparent plastic polythene paper of thickness 30 um which was weekly removed, soil mixed by use of a hoe and cover replaced till the fourth week when chemical residues was tested by planting cabbage seeds before tomato seeds were planted. Fumigation procedure adopted from [11]. Delinted tomato seeds were chemical dressed in a one- litre plastic bucket where a syringe was used to transfer liquid Gaucho at the rate of 8 ml/1kg whilst a slurry of Captan was prepared and dispersed into the seeds at 1.2 ml/kg of seeds before the mixing was done manually by shaking the plastic bucket through a 360^0 rotation for at least one minute. The recommended agronomic practices were observed throughout the growing period.

Inoculum preparation and inoculation

A mixed potato inoculum of 56 pathogenic *R. solani* was prepared by mixing 50 g of finely chopped potato with 500 cm^3 of light textured soil, placed in a one- lire flask, autoclaved for 20 minutes and cooled. Under aseptic conditions, three small mycelial agar discs obtained from the margin of each of the young colonies of *R. solani* growing on acidified potato dextrose agar plates were transferred to the mixture. Each isolate was produced in triplicate. The containers were incubated at 22-25 °C for 12 days at the end of which were all emptied and the infested soil mixed thoroughly to make soil- potato inoculum. Count per unit was adjusted to 10^6 mycelial fragments per gram and 2-4 cm of the soil-potato inoculum added to cover tomato seeds planted in a steam sterilized potting soil mixture in the greenhouse. For field inoculation, 200 cm^3 of *R. solani* potato soil inoculum per hill was used and mixed with the soil gently before seeds were planted. Procedure adopted from [12, 13, 14].

Disease and yield assessments

Each tomato variety was evaluated independently and the mean for the three varieties calculated for each treatment. Damping off incidence was assessed every five days for 5 to 30 DAP by counting the number of emerged but dead seedlings or those exhibiting the root rot/wire stem symptoms per replicate. All the replicates were tallied and finally the cumulative percentage survival of the total planted per treatment calculated. Damping off severity was estimated at the end of 30 DAP by destructive sampling of 12 plants per replicate from which the mean value per treatment was calculated. After washing the lower stem and roots off soil under running tap water, the scale of 1-4 was used to categorize the various infection levels as; 1=no disease/healthy seedling, 2=diseased seedling, 3=post emerged damped-off/dead seedling and 4=pre-emergence damped-off seedling. Crop stand was determined at 60 DAP by counting the number of established plants per replicate after which percentage stand per treatment was calculated. Disease assessment protocol adopted from [12, 14].

Yield assessment was done at maturity of the plants by harvesting, grading and weighing all the marketable fruits per replicate and mean per treatment block converted to yield in tons per hectare. The fruit quality was assessed on a grading system of GI-III based on size and weight of fruit as follows; GI=≥3-4 cm diameter and ≥80 g/fruit, GII=2-2.9 cm diameter and 59-79 g/fruit, GIII=≤2 cm diameter and ≤ 60 g/fruit. Each grade per treatment was expressed as percent of the total. Grading procedure adopted from [4]. Where the cultural practices were evaluated singly, the data for individual levels of cultural strategies were considered (Table 1-4) whilst for combination of the strategies the mean for various levels was taken (Table 5).

Statistical data analysis
At the end of the two experiments all the data collected was analyzed by One Way ANOVA using Gen start 6th edition software and means separated using fisher's least significance difference (LSD) procedure at P≤0.05. In case of zero values on the data of percent seedling survival, transformation to respective arc sine values was done before analysis. Procedure adopted from who? Author to provide [15].

RESULTS

Efficacy of cultural methods in management of Rhizoctonia damping off of tomato
Planting tomato on raised beds increased (though not significantly at P≤0.05) the percent seedling survival, decreased damping off severity, increased crop stand, and increased both quality and quantity of yield as compared to flat beds (Table 1).

Manure application at 150 and 300 cm^3/hill resulted in significantly reduced percent seedling survival, increased disease severity, reduced crop stand and lowered quantity but not quality of yield as compared to plots with no cow manure. Increasing manure application from 150 to 300 cm^3/hill resulted in decrease (though not always significant) in percent survival of seedling, increase in damping off severity, decrease in crop stand and decrease in yield (Table 2).

Watering the plots normally or daily increased infection by *Rhizoctonia* as indicated by lower percent seedling survival, higher damping off incidence and severity, lower crop stand and lower quality and quantity of yield. Further increase in irrigation interval to alternate and finally to after two days not only resulted in lower damping off severity but also in poor seedling survival and stand, and depressed quality and quantity of yield. However, watering alternate days produced the best compromise on percent seedling survival, disease severity, crop stand and yield (Table 3).

Transplanting resulted in significant increase on percent tomato seedling survival at 25 and 30 DAP, decrease in damping off severity, increased in percent crop stand and higher quality and quantity of yield as compared to direct seeding (Table 4). For all the cultural practices, transplanting had the greatest impact on Rhizoctonia damping off of tomato control, producing lowest disease severity, highest percent seedling survival (>70%) and yield that in most cases were not significantly different from fumigation with Basamid and chemical seed dressing with Gaucho. However, fumigated plots produced excellent disease control as indicated by highest percent seedling survival, lowest damping off severity, and highest crop stand and yield quantity as compared to all other treatments. For seed dressing chemicals, Gaucho was superior to Captan on all disease control parameters. Transplanting, chemical seed dressing with Gaucho, raised beds, no manure application, raised beds + no manure + watering alternate days and raised beds + no manure produced moderate disease control as indicated by average percent seedling survival, crop stand and yield

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and were not significantly different from each other. Watering alternate days, watering alternate days + no manure, raised beds + watering alternate and seed dressing with Captan produced poor disease control as indicated by lower percent seedling survival, crop stand and yield, and were not significantly different from each other. In most cases treatments with high percent seedling survival had also high crop stand and consequently high mean yield but no direct relation between disease severity and other parameters was observed (Table 5).

DISCUSSION

Effect of soil moisture on Rhizoctonia damping off of tomato

In this study, planting on raised beds resulted in better drainage hence reduced infection by *R. solani* and consequently increased yield as compared to planting on flat beds. Watering on alternate days at average temperature of 22-26 ^{0}C on loam to clay soils produced the best compromise on *Rhizoctonia* infection and yield. This is in agreement with research data from Burke *et al*, and University of California Agriculture and Natural Resources [16, 17] which reported that planting in high and well drained beds, shallow planting, irrigating up to 0.05 inch and high temperatures of 16-21 ^{0}C reduced soil moisture levels hence controlled diseases caused by *Rhizoctonia spp.* However, further increase in irrigation interval to alternate and finally after two days resulted not only in lower damping off severity but also in poor germination, smaller bush and blossom end rot of fruits; which greatly depressed quality and quantity of yield thus consequently cancelling the gains brought by reduced infection. Soil moisture not only affects *Rhizoctonia* multiplication and dispersal but also seed germination and emergence, plant growth and thus yield [12, 16].

Effect of cow manure on Rhizoctonia damping off of tomato

Cow manure application at 150 and 300 cm^3 /hill increased *Rhizoctonia* infection in tomato and reduced yield. *Rhizoctonia* is a non-specialized soil borne pathogen that rapidly colonizes decomposed soil organic matter hence the natural population of the fungi in the soil in form of sclerotia and mycelia are associated with decomposed soil organic matter [12, 18]. However, most of the fruits from plots with manure were of high grade as opposed to those without perhaps due to the fact that manure acts as a fertilizer in its own right and also improves soil structure and water holding capacity. Most farmers in Kenya use cow manure as fertilizer but in *Rhizoctonia* infested fields, the benefit from soil fertilization with manure is overtaken by the fact that manure promotes *Rhizoctonia* infection. When using organic amendments, their level of decomposition and source should be considered in *Rhizoctonia* infested soils as fully decomposed material will promote *Rhizoctonia* infection whereas fresh organic matter will reduce the disease [12, 18, 19, 20]. Utilization of nitrogen and Carbon present in the organic matter by microorganism promote their populations' growth which together with organic matter decomposition products such as ammonium and carbon dioxide may play a role in pathogen decline through biocontrol mechanisms such as mycoparasitism, antibiosis and competition [20, 21].

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Efficacy of Transplanting in the control of Rhizoctonia damping off of tomato
Raising seedlings in *Rhizoctonia* free nursery and taking them to the infested field after 21 DAP significantly decreased damping off disease, perhaps because the seedlings are exposed to the inoculum when resistance has already set in as compared to the direct seeding. Similar information is available on the differential susceptibility of seedlings to Rhizoctonia damping off with age [13, 22, 23, 24]. Demerits of transplanting include creation of wounds for pathogen entry at root tips, transplanting shock and reduced yield from many old seedlings of over 21 days. This facilitation of entry by *Rhizoctonia* probably explains why there was relatively higher level of infection in transplanted seedlings as compared to direct seeded after 30 days as supported by findings from Anderson and Damping off Wikipedia [1, 25]. However, in this study, the demerits of transplanting were overtaken by the large gains from resistance seedlings resulting in increased crop stand and consequently higher yield. About 80.0% of the tomato growers in Kenya use transplanting, whereby they transplant seedlings at 18-22 DAP instead of direct seeding to save on costs of raising seedlings. However, even in transplanting, seeds are first direct seeded in the nursery, meaning it is not a complete solution to *Rhizoctonia* infection.

Comparing efficacy of cultural strategies with chemical fumigation and seed dressing in the control of Rhizoctonia damping off of tomato
All the cultural practices tested singly and in combination had direct or indirect impact on Rhizoctonia damping off incidence and severity thus affecting crop stand, disease levels and yield. These practices influenced the disease levels by influencing the level of biological activity and pH, drainage, compaction and structure, level of resistant to disease, development and vigor. Fumigation with metham sodium had excellent results in the control of Rhizoctonia damping off of tomato and resulted to highest yield perhaps because both soil solarisation and chemical soil fumigation which both control Rhizoctonia diseases in their own rights were involved. This is corroborated by reports from other researchers indicating control of soil borne pathogens by solarisation and metham sodium [27]. The soil temperature under the plastic rose to a maximum of 50^0C. Metham sodium has been successfully used as an alternative to methyl bromide in the control of *R. solani* and other soil borne pathogens in tomato production [28]. Basamid/Dazomet breaks down into bioactive agent methyl isothiocyanate (MIT) after application to moist soil which controls a broad spectrum of soil borne pests, its relatively safe to user and a non-ozone depletory [29]. However, the large quantities required, its dependency on irrigation water for activation, potential for ground water pollution from leaching and the biological vacuum created by such a high efficacy especially where water or seeds are contaminated with the pathogen are main demerits [7, 30]. Effect of the seed dressing fungicides in suppressing *Rhizoctonia* infection depended on active ingredient of the product. Gaucho MT 390 FS which contains pencycuron 50g/L, thiram 107g/L and imidacloprid 233 g/L produced good disease control whereas Captan 800 EC which contains captafol was poor. Differential control of *R. solani* isolates in the greenhouse by pencycuron and not by captafol has been reported [26]. The differential efficacy of various seed dressers against *Rhizoctonia* isolates suggests that a combination of active ingredients or seed dressing with other tactics such as raised beds or

transplanting are required for the disease control. The low level of infection reported in the uninfested soil was probably due to low levels of pathogenic *Rhizoctonia* strains and other root rot pathogens naturally occurring in the field.

CONCLUSION

Transplanting, planting on raised beds and medium irrigation interval when used singly and in combination with other cultural methods produced good disease control and higher yield that compared favorably to the conventional disease control methods by chemical soil fumigation and seed dressing. This indicates a potential of these non-chemical strategies in the control of Rhizoctonia damping off of tomato. However, despite all advantages, it is prudent to appreciate that negative side effects exist with all methods of soil disinfestations including incompatibility and inconsistence returns which may prevent wide adoption of the methods documented in this study. Future improvements of these cultural strategies through continued research, including use of molecular diagnostic techniques and strong linkage between research and extension to ensure faster adoption are required.

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Table 1: Percent seedling survival, damping off severity, crop stand, quality and quantity of yield of *R. solani* infected tomato from raised beds

Treatments	Percent seedling survival (DAP)						Disease	Percent	Quality of yield			Quantity of
	5	10	15	20	25	30	severity	crop stand	GI	GII	GIII	yield (T/ha)
Raised beds	45.1a	80.9a	82.1a	78.6ab	75.0ab	68.5ab	2.7a	66.3b	25.6a	48.6a	28.8ab	57.5ab
Flat beds	40.7ab	72.3ab	73.1ab	68.5ab	66.2ab	64.3ab	3.0a	61.5ab	27.0a	44.2a	28.4ab	49.3b

Values in the same column followed by similar letters of the alphabet not significantly different according to Duncan's multiple range test at P≤0.05; data are means of two seasons and four replicates each season. GI-III=Tomato grades I-III

Table 2: Percent seedling survival, damping off severity, crop stand, quality and quantity of yield of *R . solani* infected tomato from various levels of cow manure

Treatments	Percent seedling survival (DAP)						Disease	Percent	Quality of yield			Quantity of
	5	10	15	20	25	30	severity	crop stand	GI	GII	GIII	yield (T/ha)
*Manure 0 cm^3	40.7a	72.3a	73.1a	68.5a	66.2a	64.3a	3.0c	61.5a	27.0b	44.2a	28.4a	49.3a
*Manure 150 cm^3	35.2b	67.1ab	66.8b	62.3b	59.0b	57.0b	3.3b	53.6b	39.8a	41.0b	19.1b	44.6b
*Manure 300 cm^3	37.0b	64.0b	61.9c	59.0b	55.0b	53.7b	3.5a	50.2b	44.0a	45.9a	10.0c	44.0b

*Manure=cow manure
Values in the same column followed by similar letters of the alphabet not significantly different according to Duncan's multiple range test at P≤0.05. GI-III=Tomato grades I-III

Table 3: Percent seedling survival, damping off severity, crop stand, quality and quantity of yield of *R. solani* infected tomato from various levels of watering regime

Treatments	Percent seedling survival (DAP)						Disease	Percent	Quality of yield			Quantity of
	5	10	15	20	25	30	severity	crop stand	GI	GII	GIII	yield(T/ha)
Water normally	40.0a	81.6a	83.9a	70.2a	65.4a	62.0a	3.3a	60.0a	33.3a	46.8a	19.8d	52.3a
Water daily	34.3a	52.9b	66.0b	64.7b	63.0a	58.4a	2.5b	54.7b	21.2b	46.7a	32.0c	50.5a
Water alternate days	18.9b	30.4c	48.0c	58.3c	56.0b	50.0b	2.1b	47.8c	10.2b	32.8b	57.0b	43.0b
Water every 2 days	0.03c	7.0d	33.7d	30.0d	29.9c	27.6c	1.4c	28.2d	2.7b	15.0c	82.1b	21.9c

Values in the same column followed by similar letters of the alphabet not significantly different according to Duncan's multiple range test at P≤0.05; data are means of two seasons and four replicates each season. GI-III=Tomato grades I-III

Table 4: Percent seedling survival, damping off severity, crop stand, quality and quantity of yield of *R. solani* infected tomato from transplanting

Treatments	Percent seedling survival (DAP)						Disease	Percent	Quality of yield			Quantity of
	5	10	15	20	25	30	severity	crop stand	GI	GII	GIII	yield (T/ha)
Transplanted	0.0b	0.0a	0.0b	0.0c	85.0b	78.0a	1.8b	75.4a	26.6a	30.3b	43.0a	60.0a
Direct seeded	40.7a	72.3a	73.1a	68.5b	66.2b	64.3b	3.0a	61.5b	27.0a	44.2a	28.4b	49.3b

Values in the same column followed by similar letters of the alphabet not significantly different according to Duncan's multiple range test at P≤0.05; data are means of two seasons and four replicates each season. GI-III=Tomato grades I-III

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Table 5: Comparing efficacy of cultural strategies with chemical seed dressing and fumigation in the control of Rhizoctonia damping off of tomato

Treatment	Percent seedling survival at 30 DAP	Disease severity	Crop stand at flowering	Yield (Ton/ha)
Raised beds	68.5b	27a	66.3b	57.5b
*Manure 0 cm^3	64.3b	3.0a	61.5bc	49.3bc
Water alternate days	50.0c	2.1b	47.8d	43.0c
Transplanting	78.0ab	1.8bc	75.4b	60.0ab
Raised beds + *Manure 0 cm^3	66.4b	2.9a	63.9c	53.4bc
Raised beds + watering alternate days	59.3c	2.4ab	57.1cd	50.3bc
Watering alternate days+ *manure 0 cm^3	57.2bc	2.6ab	54.7cd	46.2c
Raised beds + *manure 0 cm^3 + watering alternate days	60.9bc	2.6ab	58.5bc	49.9bc
Basamid 800 Gr	93.0a	1.0c	90.5a	69.9a
Gaucho 390 MT FS	81.5ab	1.6bc	79.0a	62.0ab
Captan 1200 EC	58.0c	2.7a	53.0c	50.6bc
Uninfested	84.0a	1.2c	82.0a	65.0a

*Manure=cow manure

Values in the same column followed by similar letters of the alphabet not significantly different according to Duncan's multiple range test at P≤0.05; data are means of two seasons and four replicates each season

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