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Compatibility of Predatory Mite (*Amblyseius californicus*) with Entomopathogenic Fungi against Two Spotted Spider Mite (*Tetranychus urticae*. Koch)

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Abstract

*The two-spotted spider mite (***Tetranchus urticae***) is the most threatening pest of flower farms in Ethiopia. Biological control is becoming an acceptable practice to suppress the pest in greenhouse. Recently reported to control spider mite is a predatory mite native to Ethiopia. However, information on its ability to combine with other control measures is inadequate. This study was carried out to investigate its compatibility with two entomopathogenic fungi against* **T. urticae** *(Koch) under laboratory and glasshouse condition. Four fungal isolates: 9609, GG, MM and PPRC6 with four levels of conidial concentrations: 10^6, 10^7, 10^8 and 10^9conidia/ml were used as treatments.* **Amblyseius californicus** *motiles were recorded at four, six and eight days after application. Among conidial concentrations tested, MM x 10^7 and MM x 10^8 conidia/ml are showed no detrimental effect on A.californicus during all exposure time compared to others higher conidial concentrations during laboratory trial; thus they were combined with* **A.californicus** *to check their effect against* **T. urticae** *in greenhouse condition. Alive mite's motiles were recorded for data analyses. Under greenhouse, during 6^{th} and 8^{th} days MM x 10^7 conidia/ml + 5:50 (***A.californicus: T. urticae***) ratio reduced numbers of T. urticae population by 44.37 and 43.33% respectively compared to untreated (0.01% Tween 80 solution alone) while population of* **T.urticae** *was reduced by 21.6 and 22.83% at* **A.californicas** *alone compared to untreated control. Therefore, from the present study the combination of MM x 10^7 conidia/ml + 5:50 (***A.californicus: T. urticae***) were found best for controlling spider mites populations under greenhouse conditions without adverse effect on* **A.californicus**.

Keywords: Compatibility, *A.californicus,* Entomopathogenic Fungi, and *T. urticae*

Introduction

Horticultural crops including roses are contributing remarkable economic importance to the Ethiopian society (ECA, 2007). The country has gained about 8 million USD in the year 2006 from the sale of flowers. Although flower production and exports has greatly increased in the last few years, the number and severity of disease and insect pests had also significantly increased (ECA, 2007)

Many pests have the potential to cause great destruction in greenhouse crops. Those pests which are most difficult to control are: Western

Flower Thrips (*Frankliniella occidentalis*), the two spotted Spider Mite (*Tetranychus urticae*), Rose Powdery Mildew (*Spaerotheca pannosa rosae*), mealybugs, aphids and whiteflies (Meyer, 1996). The two-spotted spider mite is an important arthropod pest that reduces ornamental and food crops production throughout the World. It is generally a foliage feeder that extracts the cell contents from leaf parenchyma tissue, causing foliar stippling, disruption of the plant's photosynthetic process, water balance mechanisms and severe infestation can also damage the flower bud (Meyer, 1996). High mite levels had been reported to cause unattractive webbing which reduces the quality of marketable product (Lieth *et al*., 1999 and Dreistadt, *et al*., 2001). The application of insecticides to control insects reduced natural enemies, thereby causing a decline in predation pressure, which allowed the two-spotted spider mite population to increase (Gerson and Cohen, 1989).

Biological control agents such as entomopathogenic fungi, and predatory mites (Phytoseiidae), has become a more acceptable practice in greenhouse environment (Skirvin *et al*., 2002; Shi and Feng, 2004; Roobakkumar *et al*., 2010). Today, among predatory mite, *Amblyseius californicus* is found best suited to crops of long duration or as a preventive measure for the long term control of green house spider mite population. It has been reported to be more tolerant of higher temperature, lower relative humidity and the use of chemical pesticides (Blumel and Walzer, 2002), even as its ability to combine with other bio-agent to control mites and other insect pest has also been observed (Castagnoli and Simoni 1991). However, there are occasions when the activity of *A.californicus* breaks down, usually due to biological characteristics of the predatory mite. To solve such problems, using other best compatible control options is the best strategy. Entomopathogenic fungi including *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) (as reviewed by Sunderland *et al*., 1997) are the most used and frequently combined with other bio-agent for the control of pest population. They have contact action, work quickly, and can be applied to the crop using conventional spray apparatus. Earlier studies showed that *B. bassiana* preparations were compatible with several chemical and predatory mites. (Wang J *et al*, 2011) reported *Beauveria bassiana* isolate RSB combined the predator *N.barkeri* has potential to control *F.occidentalis* under field condition without no adverse effects on this predator. Similarly, *B.bassiana*, strain SZ-26 has been identified as one of the most virulent strains against *F. occidentalis* while it displayed no virulence to *N.barkeri* (Wu *et al*.2014). The compatibility of *B. bassiana* with the predatory mite *Amblyseus sp*. (Oudemans) under laboratory and glasshouse conditions on cucumbers showed no adverse effects on the predatory mite population (Jacobson

et al., 2001). Therefore, the study investigate compatibility of locally collected predatory mite with entomopathogen fungi to control two spotted spider mite under laboratory and greenhouse conditions.

Materials and Methods

Description of the study area

The Laboratory studies were conducted at Ambo Plant Protection Research Center (APPRC) in 2011/12. APPRC is located at Ambo N 8°57′ and 38°07′E western Ethiopia with an altitude of 2195 m.a.l, and 110 km away from Addis Ababa. It has a mean annual rain fall of 1115 mm and mean minimum and maximum temperatures of 11.7°C and 25.4°C, respectively. The center is equipped with a greenhouse and laboratory appropriate for spider mite and predatory mite rearing.

Collection and Rearing of Mites

About 300-500 two Spotted Spider Mites (TSSM) were collected from Ethiodream PLC rose greenhouse at Addis Alem Floriculture and reared on haricot bean (*Phaseolus vulgaris*) (Awash Melka variety) grown in cages with 1 x 1 x 1.3m size. The predatory mite was collected from around Sabata and its vicinity and reared in green house by supplying spider mites as a food. Rearing process was maintained at temperature 23-25°C, photoperiod of 12:12h light and dark and a relative humidity of approximately 60-70%. Hand lens and simple microscope were used to identify both the host and the bio-agent throughout the experimental period. Reared mites were used as stock to carry out experiments.

Fungal Isolates used: Four isolates of *Beauveria bassiana* and *Metarhizium anisopliae* collected from different agro-ecological zones from different arthropods were taken from Ambo Plant protection Research Center (PPRC) (Table 1). The fungal Isolates had been screened by the PPRC against a variety of insect pests and their potential effectiveness were proven and recommended for use by the center.

Table 1: Fungal isolates tested with *A.culifornicus* compatibility against *T.urticae*

Species	Isolate Code	Location collected	Origin
Metarhizium anisopliae	MM	Arbaminch	From soil
	PPRC6	Kewot (N.Shoa)	*Pachnoda interrupta*
Beauveria bassiana	9609	Mugundo (Dila road)	*Blosyrus rugulosus*
	GG	Ashenge (Tigray)	Coleoptera(Adult)

Source: PPRC/EIAR, Ethiopia, 2000

Laboratory trial

Fungal Isolates and Conidial Preparation

The initial cultures of all isolates were stored in the center as conidial form at 4 ^{o}C. The isolates were cultured on sabouraud dextrose agar (40g dextrose, 10g mycological peptone and 15g agar in one liter of distilled sterilized water and 2g yeast extract (SDAY). Malt extract agar with *B. bassiana* and *M.anisopliae* isolates were inoculated onto SDAY containing petridishes with originally stored conidia, in powder form under refrigeration at 4^{o}C. These petridishes were closed with parafilm to reduce dehydration and maintain adequate moisture levels for fungal growth. The inoculated materials were kept at 27^{o}C in the dark for 15days. Then after this time, they were removed from the incubator and conidial suspensions were prepared by harvesting fungal conidia using sterile scalpel and added in 10ml sterile distilled water containing 0.01% Tween 80 test tube. The transferred conidia were shaked rapidly for one minute to break up conidial clumps and the mycelia were separated from pure conidial suspensions using the spatula. The suspension was diluted to 10 times by making serial dilutions. The suspension was then adjusted to targeted concentrations based on the counts of conidia, in one of suspension, made with improved Neubauer haemocytometer under compound microscope (40 x magnifications). The original suspensions were then diluted to obtain final concentrations. The viability of the conidia was confirmed according to the method of Wen *et al*. (2003).

Fungal suspension and inoculation

Experiments were done at adjusted temperature 27^{o}C and about 80 % Relative humidity under laboratory conditions. Infested bean leaves were collected and kept in a refrigerator at 4^{o}C overnight to stop the mobility of *A.californicus*. The *A.californicus* was counted under binocular microscope and individuals transferred to a single bean leaf using an insect pin, placed on filter paper, inside a petridish (12.5cm diameter). For each petri dish 10 *A.californicus* were treated by fungal isolates. Four fungal isolates: 9609, GG, MM and PPRC6 with four levels of conidial concentrations: 10^{6}, 10^{7}, 10^{8} and 10^{9} conidia/ml and untreated control were inoculated against *A.californicus* in petri dish. *A.californicus* motiles were recorded

from each petri dish for data analyses. Complete randomized design (CRD) with three replications was used. Adult *T.urticae* was supplied as food.

Greenhouse trial

The experiment was conducted on bean plants. Plants were grown in pots (20 x 19cm) and placed in the cage with the size of 1 x 1 x 1.3 m. A total of 24 pots were used for this trial. Concentration of MM x 10^7 and MM x 10^8 conidia/ml had no adverse effect on population of *A.californicus* during all exposure time compared to others higher concentrations during laboratory experiments were selected for further study in greenhouse condition. Bean Plants of one week old were released with 50 adult *T.urticae* + 5 adult *A.californicus* to each plot one week before fungal pathogen application for experimentation. As a result (MM x 10^8 conidia/ml + *A.californicus*), (MM x 10^7 conidia/ml + *A.californicus*), and (*A.californicus* alone) and untreated control were used as treatment to check their combined effect against *T. urticae*. Conidia were formulated in a 0.01% Tween 80 solution. The adjusted Conidia were sprayed on the plants after releasing the mites using a 500ml hand sprayer. The experiment was conducted in randomized complete block design (RCBD) with six replications. Conidia were formulated in a 0.01% Tween 80 solution.

Data collection and analysis

During data collection from each pot 10 leaflet were sampled and all motiles (larvae, nymphs, and adults) stages of mites were recorded before fungal pathogen application and both mites motiles at 4^{th} , 6^{th} and 8^{th} days after fungal pathogen application using dissect microscope. The average numbers of A. Californicus and T.urticae motiles percents were calculated. Temperature and relative humidity were recorded throughout the experimental work.

The efficacy of the tested treatments was calculated from greenhouse data according to the following formula described by Henderson and Tilton (1955).

Treatment Efficiency (%) = [1-(t1 x c1 / t2 x c2) x 100] Where t1 = number of Motile stages on the treated leaf after treatment, t2 = number of Motile stages on the treated leaf before treatment, c1 = number of Motile stages on the control leaf after treatment, and c2 = number of Motile stages on the control leaf before treatment.

Leaf damage assessment was made using a scale of 0-5; 0 = no damage, 1 = 20%, 2 = 40% 3 = 60% , 4 = 80% and 5: 100% following Kondo, (2004) methodology based on the lower leaf surface spotted were calculated at five days intervals starting from the 18^{th} to 28^{th} days after treatment.

Data were analyzed using (SAS institute 1988) to determine its significance. Means were compared using Least Significant Difference at $P \leq 0.05$ probability level.

Results and Discussion

Laboratory Bioassay:

The Effect of Fungal Concentrations on *A.californicus*

The result presented in (Table 2.) showed that *A.californicus* has different response to fungal concentrations. At 4th days after application of fungal concentration such as 9609 x 10^6, GG x 10^6, MM x 10^6, MM x 10^7, MM x 10^8, PPRC6 x 10^6, and PPRC6 x 10^7 conidia/ml, the mean number of *A.californicus* motiles was 8.0, 8.0, and 7.7, 6.7, 6.7, 8.0, 7.3 respectively. These concentrations showed no significant difference when compared to untreated control (Tween solution alone) ($P < 0.001$). At 6th days after application, GG × 10^7, MM × 10^6, MM × 10^8 and PPRC6 × 10^6 conidia/ml showed a non significant difference when compared to the untreated control (Table 2). Moreover, GG x 10^6, MM x 10^7, and MM × 10^8 conidia/ml were observed to be non significant at eight days after application when compared with untreated control. The result showed that, except for MM x 10^7 and MM x 10^8 conidia/ml, higher conidial concentrations resulted to low *A.californicus* motiles. MM x 10^7 and MM x 10^8 conidia/ml showed no detrimental effect on *A.californicus* during all exposure time compared to others higher concentrations during laboratory trial (Table 2). Alves *et al.* (2002) had reported low numbers of *A.californicus* motiles as concentration of fungal conidia increases. Furlong & Pell (1996) reported that in their study of compatibility between entomopathogenic fungi at higher concentration with predator mites had negative interactions in resource competition, infection of predators by fungi, and the predation of fungal-infected hosts. In contrast to this, current finding showed that high percent mean of *A.californicus* motiles were recorded at MM x 10^7 and MM x 10^8 conidia/ml level of concentrations in all intervals of days. This different result recorded probably caused by biological trait of bio-agents, mode of application and time of exposure. This has similar with the report of Jacobson *et al*., (2001), which state that the combination of *Amblyseius cucumeris* (Oudemans) with *B. bassiana* at higher concentration had no significant effect on this predator mite to control *Frankliniella occidentalis* (Pergande) in a commercial crop (Jacobson *et al*., 2001). Wu *et al.* (2014) also reported that in their laboratory study, strain SZ-26 was identified as one of the most virulent strains against *F.occidentalis* adults while it displayed no virulence to *N.barkeri*.

Table 2: Mean (±SE) motile's of *Amblyseius culifornicus* after fungal applications

No	Treatments (conidia/ml) (conidial ml^{-1})	Mean alive(±SE) after applications		
		4th days	6th days	8th days
1	9609 × 10^6	8.0±1.00ba	5.7±0.58bc	4.3±0.58fedc
2	9609 × 10^7	6.3±0.58bc	4.0±1.00de	3.7±1.15fe
3	9609 ×10^8	2.7±0.58d	1.0±1.00g	0.67±0.58g
4	9609 ×10^9	3.0±1.00d	1.3±0.58gf	0.67±0.57g
5	GG × 10^6	8.0±0.00ba	5.3±0.58c	5.0±0.58bac
6	GG × 10^7	7.7±1.15bac	6.67±0.58ba	4.7±0.57bcd
7	GG × 10^8	4.0±1.00d	1.3±0.58gf	1.0±0.0g
8	GG × 10^9	6.3±0.58c	1.7±0.58gf	1.67±0.58g
9	MM × 10^6	7.7±1.15bac	7.3±0.58a	4.0±1.00fed
10	MM × 10^7	6.7±1.53bac	6.0±1.00ba	5.0±0.00bac
11	MM × 10^8	6.7±0.58bac	6.5±1.15ba	4.67±0.57bac
12	MM ×10^9	3.3±0.58d	0.7±0.57g	1.3±0.58g
13	PPRC6 × 10^6	8.0±0.00ba	7.0±1.00a	4.3± 0.58fedc
14	PPRC6 × 10^7	7.3±0.58bac	5.0±1.00dc	3.67±0.57fe
15	PPRC6 × 10^8	6.3±0.58c	3.7±0.58e	3.3 0.57f
16	PPRC6 x 10^9	4.0±1.00d	2.3±0.58f	3.3±0.58f
17	DW	8.7±0.58a	7.7±0.58a	6.3 ± 0.5ba
	CV (%)	13	18.02	18.04
	LSD (0.05)	1.3746	1.273	1.04
	F Value	17.07	30.57	24.76
	P Value	P<0.001	P<0.001	P<0.001

Means followed by the same letters within Columns are not significantly different at P<.0.05 level of probability.

Greenhouse Trial

Effects of fungal concentration on predatory mites (*A.californicus*)

There was no significant treatments effect on the mean percent of *A.californicus* motiles at 4 days after fungal application (Table 3). The mean percent of *A.californicus* motiles recorded at 6th and 8th days were not significantly different between *A.californicus* alone and *A.californicus* plus MM x 10^7 conidia/ml (P<0.001), however, there was a significant difference observed between *A.californicus* plus MM x 10^8 conidia/ml (Table 3). At six and eight days treatments *A.californicus* plus MM x 10^8 conidia/ml showed statistically low percent motiles of *A.californicus* compared to other treatments (Table 3). Even though, most studies suggest that combination of two bio-agent cause adverse effect on each other. For example, according to Fransen and van Lenteren (1994) had reported that, the separate applications of entomopathogenic fungus (Aschersonia *aleyrodis* Webber) and the parasitoid (*Encarsia formosa* Gahan) was more effective than their combination for the control of *T. vaporariorum.* This has been attributed to reduced competition between these natural enemies. However, some researchers disagreed on the screening of natural enemies and releasing only the one most effective

species (Briggs 1993; Ehler and Hall 1982). In line with their finding, our glasshouse and laboratory bioassay studies were consistent because some of the concentrations did not killed *A.californicas* during laboratory and greenhouse conditions (Tables 2 and 3).

The greenhouse result was also in agreement with Bonaventure *et al. (*2012) who studied interaction of entomopathogenic fungus and predatory mites to control cassava green mites.

Table 3. Percentage mean motile of *A.culifornicus* observed after application of fungal concentration in Green House Conditions

Nos	Treatments	Percentage (%) mean *A.californicus* motile after treatments		
		4th days	6th days	8th days
1	5:50 (A.c:T.u)	80.0±2.83a	96.7±2.8a	76.7±2.2a
2	MM x 10^{8}*+5:50(A.c:T.u)	67.0±1.97a	68.3±2.32b	63.0±1.36b
3	MMx10^{7}* + 5:50(A.c:T.u)	76.7±2.40a	88.±1.72ba	86.7 ±2.07a
	LSD (0.05)	2.4596	2.65	1.12
	CV (%)	26.27	24.43	13.25

Means followed by the same letters within Columns are not significantly different at P <0.05 level of probability.
A.c = *Amblyseius. californicus* T.u = *Tetranychus. Urticae*, *= conidia/ml

Treatments Effect on Two Spotted Spider Mite (*T.urticae*) Population

After four days treatment MMx10^{7} conidia/ml + *A.californicas* and *A.californicas*- alone with mean numbers 63.67±3.56 and 68.0 ±2.83% respectively showed no statistically significant difference when compared to each other. However, these treatments showed significant difference when compared to untreated control (95.0±4.38%) and MMx10^{8} conidia/ml + *A.californicas* 41.67±4.5 % ($p > 0.001$) (Table 4). The performance of *A.californicus* in fourth days in greenhouse experiment is probably due to its ability to feed immediately on two spotted spider mite, while *M.anisopliae* requires some time to develop and spread though the mite body. This finding similar with Hajek *et al.,* (2001) studies who state that entomopathogenic fungi kill the host only after completely growing through the host's body. At six and eight days MM x 10^{7} conidia/ml + *A.californicas* and MM x 10^{8} conidia/ml + *A.californicas* reduced the population *T.urticae* when compared to *A.californicas* alone and untreated control. *M.anisopliae* mycelium growth conformed from dead *T.urticae* is also evident for the toxicity of the fungal pathogen (Figure.1). Croft *et al.,* 1992 found well evidence that two or more natural enemy species together provides higher suppression when they co-exist some time in similar ecology than single natural enemy species applied. Wu *et al.* (2014) also reported that fungal treated pests can affect the functional response parameters of predator, and in turn, the predators behavior for attacking pests could promote performance of the fungus.

In current study, low percent mean of two spotted spider mites motile's were recorded at MMx10^8 conidia/ml + *A.californicas* while more motiles were observed on untreated control. The two spotted spider mites population increased over time in the untreated control, probably because of absence of bio-agent competition (Table 4). This result was similar to (Walzer, 1999b) result who reported that a continuous release of bio-agent has a medium-term lasting effect in the management of populations of phytophagous mites. (*Wekesa et al.* 2007) also reported that interactions among natural enemy species have two opposite effects on target pest population. Likewise Maketon *et al* (2008) studied that *M. anisopliae* was capable of infecting the two spotted mites: and when combined with other bio-agent which resulted in a faster death of the two spotted spider mites, while density of the mites also affected the activity of the bio-agent. The authors concluded from their experiments that the fungus selected should cause epizootics within 2–3 days following application. Promising results for the control of the broad mite on mulberry have been obtained with *M. anisopliae.* At six and eight days MMx10^8 conidia/ml + *A.californicas* reduced population *T.urticae* by 59.21 and 49.83% respectively when compared with untreated control. In the same interval of time MMx10^7 + *A.californicas* reduced population *T.urticae* by 44.37 and 43.33 % respectively while population of *T.urticae* was reduced by 21.6 and 22.83% at *A.californicas* alone compared to untreated control. From current green house treatment the result showed that MMx10^8 conidia/ml + *A.californicas* had potential effects to reduce predator and prey percent mean population when compare to MMx10^7 + *A.californicas* combination which had significant percent population mean reduction on Spider mite while it not showed detrimental effect on *A.californicas* percent mean population (Table 3 and 4).

Table 4: Percentage mean motile *T.urticae* after application of fungal concentrations and mite ratio

No	Treatments	Percentage (%) Mean Motile of *T.urticae* (±SE) at Different Days		
		4th days	6th days	8th days
1	5:50 (A.c:T.u)	68.0 ±2.83b	87.2±2.64b	71.0±13.99b
2	MMx10^8 *x5:50 (A.c:T.u)	41.67±4.50c	50.0±1.79d	44.0±8.327d
3	MMx10^7 *x5:50 (A.c:T.u)	63.67±3.56b	64.83±3.1c	50.50±11.59c
4	Control (DW)	95.0±4.38a	109.2±8.77a	93.83±11.77a
	CV (%)	5.8	5.65	5.49
	F Value	72.58	67.67	94.43
	P Value	P<0.001	P<0.001	P<0.001
	LSD (0.05)	4.76	5.58	4.55

Means followed by the same letters within Columns are not significantly different at P≤.05 level of Probability.
A.c = *Amblyseius. californicus* T.u = *Tetranychus. Urticae*, *= conidia/ml

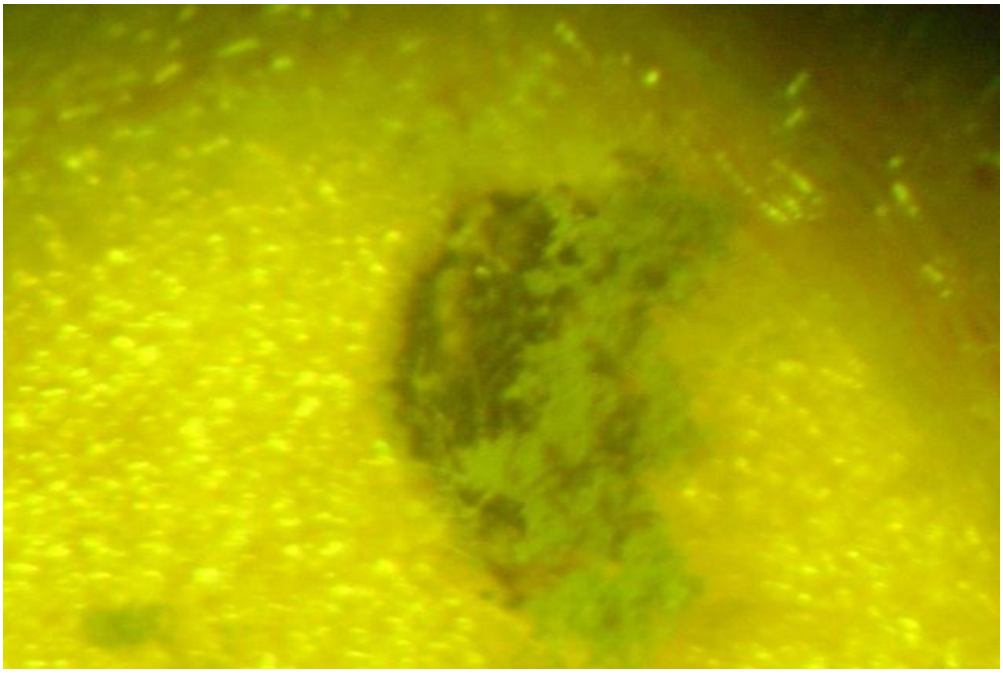

Fig.1 Mycelium growth on mite Cadaver when treated with isolate MM

Assessment of Leaf Damage

Additionally leaf damage was observed for three interval of time after 10 days of treatment application. There were significant difference between damage level of treated leaves and untreated control (.01% Tween 80) in all three intervals of days ($p \leq 0.05$). MM x 10^7 conidia/ml + *A.californicas* and MM x 10^8 conidia/ml + *A.californicas* showed no significant difference in all intervals of time while *A.californicas* alone showed significant difference in these days (Table5). In all exposure time, the total leaf damage observed on untreated were 25-43% while it reduced to 6.7-16.7 % at MM x 10^7 + *A.californicas* and MM x 10^8 conidia/ml + *A.californicas* treatments whereas, we recorded 15-25% leaf damage on *A.californicas* alone.

Table 5: Percentage mean leaf damage after 10, 15, and 20 days of treatments

No	Treatments	Percentage means of leaf damage (SE±) days after treatments		
		10	15	20
1	5:50 (A.c:T.u)	15.0+0.57b	22.0+0.75b	25.0+0.57b
2	MMx10^8*x5:50 (A.c:T.u) (A.c:T.u)	6.70±0.52c	12.0±0.41c	15.0±0.56c
3	MMx10^7*x5:50 (A.c:T.u)	8.0± 0.41c	13.0 ±0.52c	16.7±0.52c
4	Control (DW)	25.0 ±0.55a	35.0±0.84a	43.0±0.52a
	LSD (0.05)	0.7	0.76	0.88
	CV (%)	31.4	24.6	23.5

Means followed by the same letters within Columns are not significantly different at P≤.05 level of Probability.
A.c = *Amblyseius. californicus* T.u = *Tetranychus. Urticae*, *= conidia/ml

From current green house trials, the result showed that MM x 10^8 conidia/ml + *A.californicas* had potential effects to reduce predator and prey percent mean population when compare to MMx10^7 + *A.californicas* combination which had significant percent population mean reduction on Spider mite while it not showed detrimental effect on *A.californicas* percent mean population (Table 3 and 4). From current study we suggest that some wounds caused by predatory mites on Spider mite body probably serve pathogens as entry site while Spider mite infected by pathogen improve captures time of predatory mites. This finding agree with Lomer *et al.* (2001) reports in case of pathogen which state that the fungus was able to infect treated adult mites at least 4 days after inoculation and reached 90% mortality by the 8 day.

Conclusion

In conclusion biological control of two spotted spider mite in the glasshouse can be improved by combining predatory mite and Enthomopathogenic fungi rather than appling predatory mite alone. From this finding we conclude that the combination of MMx10^7 conidia/ml + *A.californicas* at 1:5 (predator: prey ratio) could provide an effective control of two spotted spider mites at green house conditions. And it is better to apply this fungal pathogen spray when two spotted mite's populations exceed the ability of *A.californicas* to control them. The frequent release of predatory mites and fungal pathogen should be further studied to determine acceptable rate and time of these bio-agents.. Further testing of the compatibility of *Metarhizium anisopliae* isolates with *A.californicas* under field condition is suggested.

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