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REARING TOMATO WHITEFLY AND FIELD EVALUATION OF MODIFIED AND UNMODIFIED CONIDIA OF *Beauveria bassiana, Isaria farinosa, Metarhizium anisopliae* AND LOW RATES OF CHLORPYRIFOS UNDER TROPICAL CONDITIONS

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ABSTRACT

The whitefly (*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a serious pest of a wide range of economically important agricultural and ornamental crops in all agro-ecological regions in the world. This study evaluated the efficacy of conidia of entomopathogenic *Beauveria bassiana* (BB 315), *Isaria farinosa* (ARSEF 5081) and *Metarhizium anisopliae* (Ma 275.86DC), against whitefly (*Bemisia tabaci)* under tropical climate. Two forms of conidia: (a) modified conidia (conidia with improved ecological competence), and (b) unmodified conidia of the three isolates were subjected to heat treatment (45°C) for 5 days. Germination rates of these conidia before and after their exposure to heat were compared at 25 °C on Sabouraud Dextrose Agar (SDA, a_w= 0.995) to assess resilience to heat stress. Further, assessment of germination potentials of conidia was conducted at three a_w levels (0.995, 0.98 and 0.96: corresponding to different levels of water availability). Thereafter, they were tested against *B. tabaci* infestation on tomato plants and insect densities after the treatments were compared with the effect of low rate chlorpyrifos. The conidia germinated differently on SDA at the three a_w levels and the rate of germination of modified conidia of each isolate was significantly higher at 0.98 a_w ($F_{2, 16}$ =0.31, P=0.001). Modified conidia of *B. bassiana* appeared more tolerant to temperature stress. All the isolates significantly reduced pest incidencerelative to control after 14 days and there was no significant difference (P>0.05) between the effect of modified and unmodified conidia against *B. tabaci* in the field. The level of control achieved using the conidia of the three fungal isolates was comparable with the chlorpyrifos treatment.

Key Words: *Bemisia tabaci*, entomopathogen, water activity

RÉSUMÉ

La mouche blanche (*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) est une redoubtable peste pour une large gamme de plantes cultivées et ornementales d'importance économique dans Presque toutes les zones agro-écologiques du monde. L'objectif de l'étude était d'évaluer l'efficacité des conidies d'entomopathogènes *Beauveria bassiana* (BB 315), *Isaria farinosa* (ARSEF 5081) et *Metarhizium anisopliae* (Ma 275.86DC), contre la mouche blanche (*Bemisia tabaci)* sous climat tropical. Deux formes de chacun des trois isolats de conidie : (a) conidie modifiée (conidie ayant une potentialité écologique améliorée), et (b) conidie non modifiée, ont été soumis à une température de 45°C pendant 5 jours. Le taux de germination de ces conidies, avant et après leur exposition à la chaleur, a été comparé à 25 °C sur un milieu de culture contenant du Sabouraud Dextrose Agar (SDA, a_w= 0.995) afin d'évaluer leur résilience face à la tension thermique. Ensuite, le potentiel de germination des conidies a été évalué à trois différents niveaux de disponibilité en eau a_w (0.995, 0.98 et 0.96). Enfin, les conidies ont été testées contre l'infestation des plants de tomate par *B. tabaci*, et les densités d'insectes après les traitements ont été comparées avec l'effet du faible taux de chlorpyriphos. Les taux de germination des conidies sur SDA variaient selon le niveau de disponibilité en eau, en plus le taux de germination des conidies modifiées était plus élevé chez avec les isolats à a_w = 0.98 ($F_{2, 16}$ =0.31, P=0.001). Les conidies modifiées de *B. bassiana* se sont révélées les plus tolérantes à la tension thermique. A l'opposé, les conidies de *I. farinosa* ou de *M. anisopliae* ont montré des taux

comparables de germination aux trois niveaux de a_w. On note une réduction significative de l'incidence de la peste après 14 jours, en reférence au control. De plus, il n'y a pas de différence significative (P>0.05) entre l'état modifié et non modifié des conidies en rapport avec leur réaction contre *B. tabaci*.

Mots Clés: *Bemisia tabaci*, entomopathogène, activité d'eau

INTRODUCTION

The whitefly (*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a serious pest of a wide range of economically important agricultural and ornamental crops in all agro-ecological regions in the world (Perring, 2001). It is especially an important pest in Nigeria (Ariyo *et al*., 2005) where ambient temperature and relative humidity are favourable to its development all year round. The insect feeds on phloem sap of plants and is responsible for the transmission of tomato leaf curl virus (Jahan *et al*., 2014; Polston *et al*., 2014). Besides disease transmission, large amounts of honeydew excreted by the insect encourage the development of black sooty mould on leaves (Palumbo *et al.*, 2000). A direct shading of leaves by the powdery coating has been reported to significantly reduce photosynthetic capabilities of crop plants, which results in economic loss (Pico *et al.*, 1996).

Control of *B. tabaci* has been based on the use of chemical insecticides (Castle *et al.*, 2014) which often result in development of resistant strains (Basit *et al*., 2013; Longhurst *et al*., 2013). In addition, problems of environmental toxicity (Kaur and Kaur, 2007) and killing of non-target insects are associated with chemicals. Thus, thereis the need for research into environment friendly and sustainable methods of management of this pest.

Several entomopathogenic fungi including *Beauveria bassiana* (Mohammed and Baha, 2014), *Isaria farinosa* (Wraight *et al*., 2000) and *Metarhizium anisopliae* (Islam *et al*., 2014) are potential biocontrol agents for *B. tabaci*. However, variation in temperature and water availability are two major factors that affect their growth and sporulation (Borisade and Magan, 2014); and these abiotic factors eventually modulate infectivity, virulence (Borisade and Magan, 2015) and secondary spread. Currently, little information is available on the level of success achieved by these biocontrol agents under field conditions in the tropics, where abiotic influences (temperature and relative humidity), may limit their efficacy.

The objective was to establish an alternative method of control of *B. tabaci* using fungal biocontrol agents instead of chemicals.

MATERIALS AND METHODS

Source of insects, fungal isolates and tomato seeds. A colony of *B. tabaci* was obtained from Teaching and Research Farm, Ekiti State University, Nigeria. The *Isaria farinosa* (ARSEF 5081) was obtained from the United States, Department of Agriculture, Agricultural Research Service (ARSEF); and *Beauveria bassiana* (315) was supplied by the Biological Control Centre for Africa, IITA Benin Republic; while *Metarhizium anisopliae* (Ma 275.86DC) was provided by Professor Dave Chandler, Warwick University, UK. The tomato seeds used for field trials (Ailsa Craig WSS 818) were supplied by Dr. Andrew Thompson, School of Applied Sciences, Cranfield University, United Kingdom.

Study sites. Pre-field studies on the isolates were carried out in Cranfield University, Agri-Food Institute Applied Mycology Laboratory Unit, United Kingdom; while the field experiments and rearing of insects were carried out on the Teaching and Research Farm of Crop Protection Unit, Ekiti State University in Nigeria. The field experiment was conducted between September and January during the dry season in 2012 and repeated during the same period in 2013.

Rearing of *B. tabaci.* Seedlings of tomato were potted and staked after 2 months. An insect net (Bio Quip Products; thread size =24 mm, light transmission = 80% and mesh size = 0.0105 x 0.0322) was used as the screen for *B. tabaci*.. The net was sewn into a cone shape and inverted

over the stake to cover the entire potted tomato plant. The loose end of the net was held firmly against the pot with rubber bands (Fig. 1).

A large colony of adult *B. tabaci* was introduced into the net for 14 days. Thereafter, the adult insects were removed by opening the net and shaking the plant. The abaxial part of the leaves was examined with a digital entomological microscope (Celestron Handheld LCD Digital Microscope) to confirm the presence of eggs. Some leaves were excised from the tomato; while 8-10 broad leaves ladened with more than 50 eggs or eggs + other developmental stages per leaf were left on the plant and covered with the net. The potted and infested tomato plant was left for an additional 2 to 3 weeks, until adult insects emerged in large numbers on the abaxial part of the tomato leaves.

Culturing and formulation of conidia. Initial conidia from 14 day-old culture of each fungi grown on SDA plates and incubated at 25 °C in the dark, were harvested with 10 ml reverse osmosis (RO) water (Thermo-Scientific, Barnstead ™ Lab Tower ™ RO) containing 0.05% Tween 80 (Borisade and Magan, 2014). Thereafter, the conidia suspension was standardized to 10^5 conidia ml^{-1}. The suspension was either inoculated on standard SDA media (a_w = 0.995) or glycerol modified SDA media (a_w = 0.98) in Petridishes (Sterilin ™ Standard 90 mm) and incubated at 25 °C in the dark for 21 days. In each case, the a_w of the media were confirmed by the use of Water Activity Meter (Aqualab Dew Point Water Activity Meter 4TE; accuracy = 0.003).

Conidia from 21 days culture were harvested as described previously using RO water containing calculated amounts of glycerol (isotonic to the a_w of the growth media; 0.98 $_{aw}$). Conidia suspension was poured into universal bottles and centrifuged at 2500 rpm for 15 minutes. The supernatant was decanted and the conidia was mixed with 5 ml skimmed milk. The conidia-milk suspension was immersed immediately in liquid Nitrogen and freeze-dried (Freeze drier Model FDB-5502) for 4 days to a moisture content of approximately 6%. Thereafter, 10 ml sterile vegetable oil (peanut oil) was added to the freeze dried conidia in the standard bottles and mixed to form a homogenous suspension which was refrigerated at 4 °C until required for bioassay.

Germination rates of modified and unmodified conidia. Germination rates were determined by spread plating 20 µl of 1.0 x 10^5 conidia ml^{-1} of the conidia formulation on glycerol modified SDA media (0.98 a_w and 0.96 a_w) and unmodified SDA media (0.995 a_w). Three sterile coverslips (MS-SLIDCV, 22 mm x 22 mm; 0.13-0.17 mm thick) were

Figure 1. Potted tomato on which *B. tabaci* was reared. The staked tomato was covered with net inverted over stakes to confine insects to the plants.

placed at separate positions (A, B and C) on each agar plate.

The Petri dishes were covered and sealed with Parafilm (Parafilm®$_M$, Bemis Comp. Inc. P7768) and incubated at 25 °C for 24 hours (Steridium i170 incubator). The set-up was replicated 3 times. Fifty conidia were counted randomly under microscope for each cover-slip field and the average number of germinated conidia plate^{-1} was calculated as:

$$\left(\frac{A+B+C}{3}\right)$$

(Borisade and Magan, 2014). A conidium with the germ tube length more than its diameter was considered as germinated. Percentage conidia germination was calculated as:

$$\frac{\textit{Number of germinated conidia}}{\textit{Total counted conidia}} \times \frac{100}{1}$$

Relative germination rates of heat stressed conidia. In order to evaluate the level of tolerance of modified and unmodified conidia to heat stress, 20 µl of 1.0 x10^5 conidia ml^{-1} was spread-plated on SDA media (0.995 a_w) using Drigalsky spatula. Three portions on each plate were covered with sterile coverslips and the Petridish was sealed with Parafilm. The plates were kept at 45 ± 2 °C for 5 days. Thereafter, the plates were withdrawn to a 25 °C environment and incubated for 24 hours. The rate of germination of the conidia was evaluated as described earlier.

Experimental design and routine maintenance. Tomato cultivar, Ailsa Craig WSS 818 was planted in the nursery and transplanted into a single row of 10 plants, spaced 30 cm between plants and 60 cm between rows in a randomised block design (RBD), with 3 replications. Weeding was done by hand and irrigation was carried out manually with a Watering Can. Bamboo stakes were used to support the plants to ensure that leaves were suspended above ground.

Virulence of conidia. Three conidia concentrations (1.0 x 10^5, 1.0 x 10^8 and 1.0 x 10^9 conidia ml^{-1}) from the formulations of each fungal isolate and Chlorpyrifos (2.3 ml liter^{-1}), were separately tested on *B. tabaci* nymphs, on tomato plants. Infested 7 weeks old potted plants were brought to the laboratory. Leaves were excised from each potted plant and discarded; while only 3 broad leaves containing nymphs were left on the stem.

The set-up was replicated 3 times and the position of 10 nymphs on each leaf was identified with a tiny pin perforation mark close to the nymph (Cabanillas and Jones, 2009). Visualisation was aided using a Handheld Digital microscope (20-2500 x magnification power). Each leaf was dipped completely into the conidia suspension for 2-3 seconds and the plant was kept under ambient conditions.

Mortality of the nymphs was recorded daily for 6 days. The control consisted of samples which were not immersed into the conidia suspension. After 24 hours, nymphs which showed signs of mycosis or discolouration (dark brown to black) under a stereo-microscope or those with uncharacteristic appearance were considered infected or dead. Uninfected nymphs had characteristic whitish and fairly translucent appearance.

Adult *B. tabaci* release and sampling. Adult *B. tabaci* previously reared on tomato were released into the field. The insects were very active during the day and responded to the slightest disturbance by moving from one plant to the other. Therefore, prior to insect release, each plot was screened with insect net along the perimeters to a height of 10 feet to minimise incidence of local migration and the sampling errors which may arise from such migratory behaviour. Thereafter, photographs of the abaxial leaf parts were taken at night in the dark when the insects were less active, using a digital camera (Nikon Coolpix L28). The pictures were downloaded as JPEG files with clear view of the insects on the leaves and the images were used for the insect count.

Insect sampling was done randomly for 7 plants per row of 10 plants, at 14 days post insect release (Five leaves plant^{-1} at 3-5 vegetal strata), at the first sampling. The second sampling was done at 21 days post insect release, and the timing of the second sampling was a day before the application of bio-insecticide treatments and

chlorpyrifos. Similar sampling was done at 2, 3, 4, 7 and 14 days after the application of the biocontrol agents and chlorpyrifos.

Application of biocontrol agents and chlorpyrifos. The modified and unmodified conidia formulations were diluted with RO water containing 0.05% Tween 80 to a concentration of 1.0 x 10^9 conidia ml^{-1}, and applied to the plants with a Mini Air compression Knapsack sprayer at the rate of 50 ml conidia formulation or chlorpyrifos per plant. The nozzle of the sprayer was directed upwards such that the abaxial part of the leaves received the spray. Ordinary RO water containing 0.05% Tween 80 and 1ml $litre^{-1}$ peanut oil was sprayed as control.

Conidia deposit per unit leaf area. Round cover slips with 2.5 cm diameter were randomly fixed on the abaxial part of leaves of 7 plants per row before the application of the conidia suspension. This was used to estimate conidia deposit cm^{-2} of leaf area. The cover slips were left for one hour to dry after which they were removed without touching the exposed surface and the conidia deposit $area^{-1}$ of the slip was determined under microscope.

Field temperature and relative humidity. Temperature and relative humidity meters (HTC-1) were randomly placed at 3 locations within the field, at plant canopy level of 30 cm above the ground. Temperature and corresponding relative humidity data were recorded every 3 hours throughout the duration of the study, in the 1st trial. During the second trial, only 6 hourly temperature and relative humidity data were recorded.

Statistical analysis. A correction for natural mortality was not done since there was no observed mortality in the control. The LT_{50} which was used to measure virulence (Cabanillas and Jones, 2009), was determined for each treatment at different concentrations of modified and unmodified conidia. The LT_{50} of the 3 different conidia concentrations of each isolate was determined from the graph of the percentage mortality against time. Percentage reduction in the adult insect density over time (D) was calculated relative to the density before treatment:

$$D = \frac{(dT0 - dTi)100}{dT0}$$

Where:

d_{T0} and d_{Ti} *were* mean adult *B. tabaci* density, i.e. number of *B. tabaci* per leaf which was estimated from the sampling conducted a day before the treatment and on the i^{th} day after each treatment. The data on the rate of germination were subjected to Analysis of Variance procedure (ANOVA). Where significant differences were found, a Posthoc analysis for separation of means was done using Least Significant Difference (LSD). The statistical software, IBM SPSS STATISTICS 21 was used for all data analysis and graphs were plotted using Microsoft Excel 2010.

RESULTS

Germination rates at different a_w. The unmodified and the modified conidia of *B. bassiana* (BB 315), *I. farinosa* (ARSEF 5081) and *M. anisopliae* (275.86 DC) germinated at differentially on SDA (Fig. 2). The mean germination of the modified conidia of each isolate was significantly higher than the unmodified at 0.98 a_w (P=0.001). However, unmodified *I. farinosa* had significantly higher germination rate (P=0.024).

Relative germination rates of heat stressed conidia. The mean percentage germination of modified *B. bassiana* conidia, which were pre-exposed to 45 °C for 3 days before testing for germination, had a significantly higher (75.3%) rate of germination than the unmodified conidia, (69.2%), (P<0.05) (Fig. 3). On the other hand, the germination rates of the modified and the unmodified conidia of either *I. farinosa* or *M. anisopliae* were not significant different (P>0.05).

Virulence of different conidia concentrations. Figure 4 compares the LT_{50} of 3 concentrations (1.0 x 10^5, 1.0 x10^8 and 1.0 x 10^9 conidia ml^{-1}) of

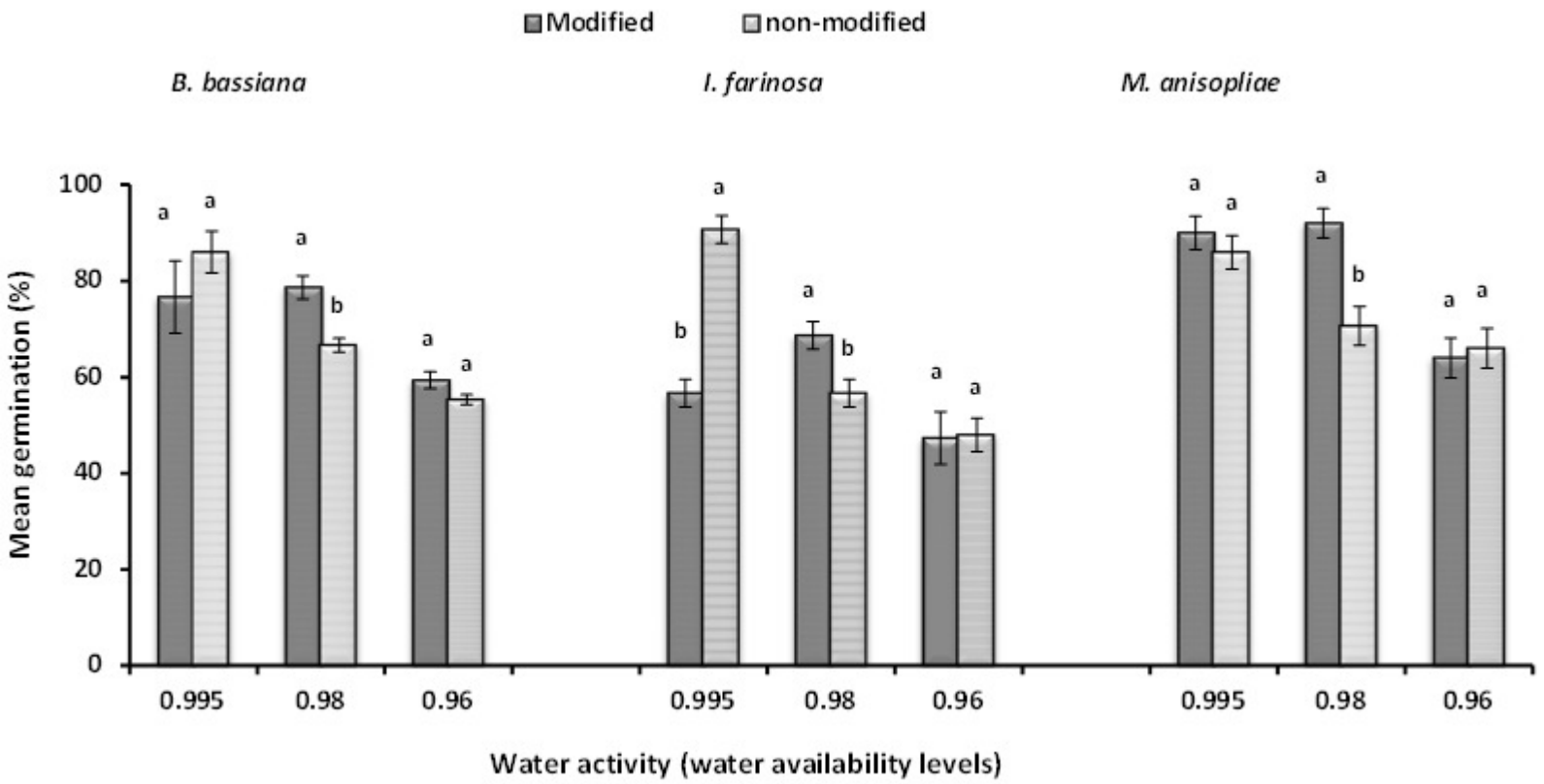

Figure 2. Mean germination of modified and unmodified conidia of 3 entomopathogenic fungi. Germination was tested on SDA at 3 different a_w levels (0.995, 0.98 and 0.96; equivalent to 3 levels of water stress) and incubated at 25 °C for 24 hours in the dark. Bars with different alphabets are significantly different ($P<0.05$).

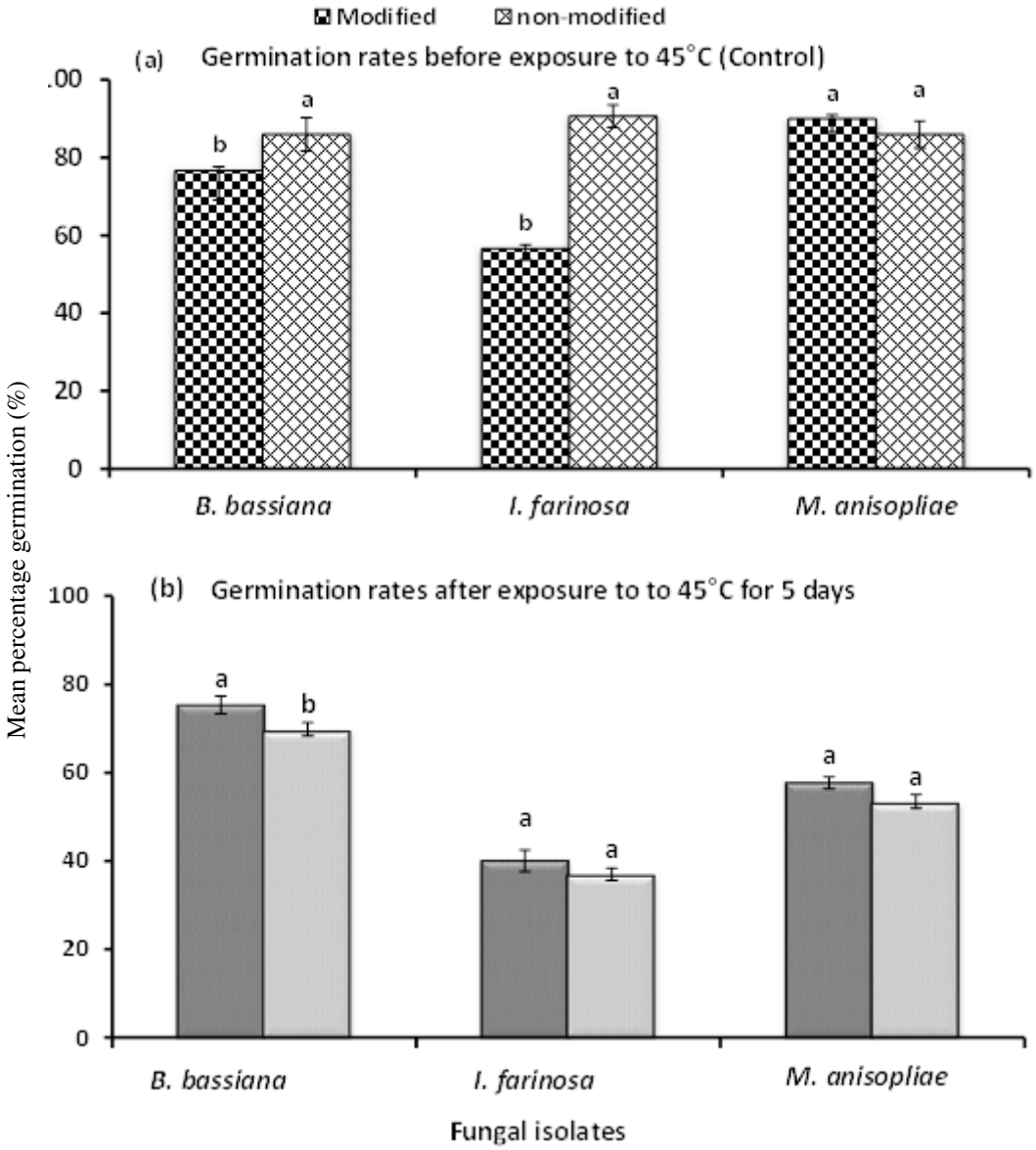

Figure 3. Mean germination of modified and unmodified conidia of 3 entomopathogenic fungal isolates: (a) modified and unmodified conidia were tested for germination on SDA at 0.995 a_w and 25 °C for 24 hours (Control); (b) the modified and unmodified conidia were inoculated on SDA (0.995 a_w) and subjected to 45 ± 2 °C for 5 days. This was followed by incubation at 25°C for 24 hours to assess the effect of the temperature treatment on germination.

modified and unmodified conidia of *B. bassiana, I. farinosa* and *M. anisopliae,* under field environmental conditions. There was no significant difference (P>0.05) in the LT_{50} of both modified and unmodified conidia of the three entomopathogenic strains. Similarly, increased concentration from 1.0 x 10^5 to 1.0 x 10^9 conidiaml^{-1} did not translate into a significantly higher virulence for the three isolates (P>0.991).

Adult *B. tabaci* population distribution. The mean number of adult *B. tabaci* per leaf and the proportion of leaves infested after 14 and 21days are shown in Figure 5. At 14 days post-release

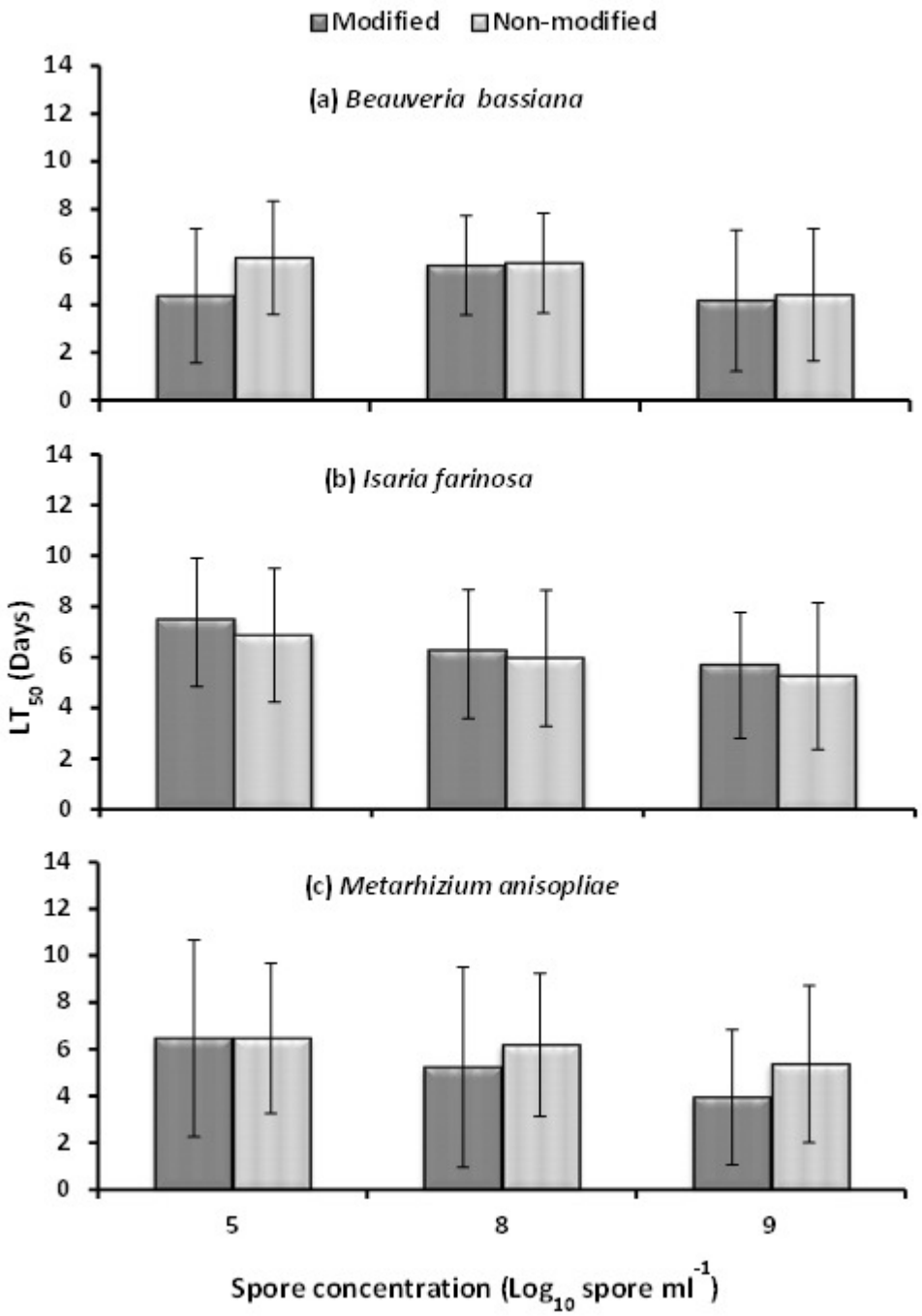

Figure 4. LT_{50} of 3 conidia concentrations (1.0×10^5, 1.0×10^8 and 1.0×10^9 conidia ml^{-1}) of modified and unmodified (a) *B. bassiana* (b) *I. farinosa* and (c) *M. anisopliae* tested against nymphs of *B. tabaci* after 6 days. Vertical bars are standard errors of the means.

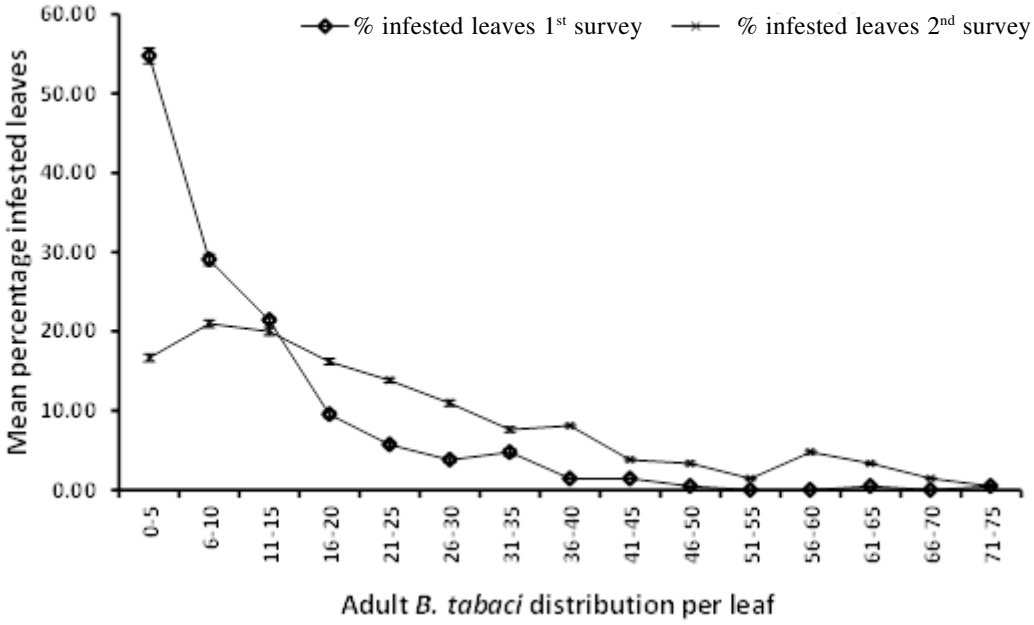

Figure 5. Numbers of *B. tabaci* per leaf corresponding to the percentage proportion of infested leaves. This represents field distribution and trends of adult *B. tabaci* population on artificially infested tomato. Bars are standard errors of the means where shown.

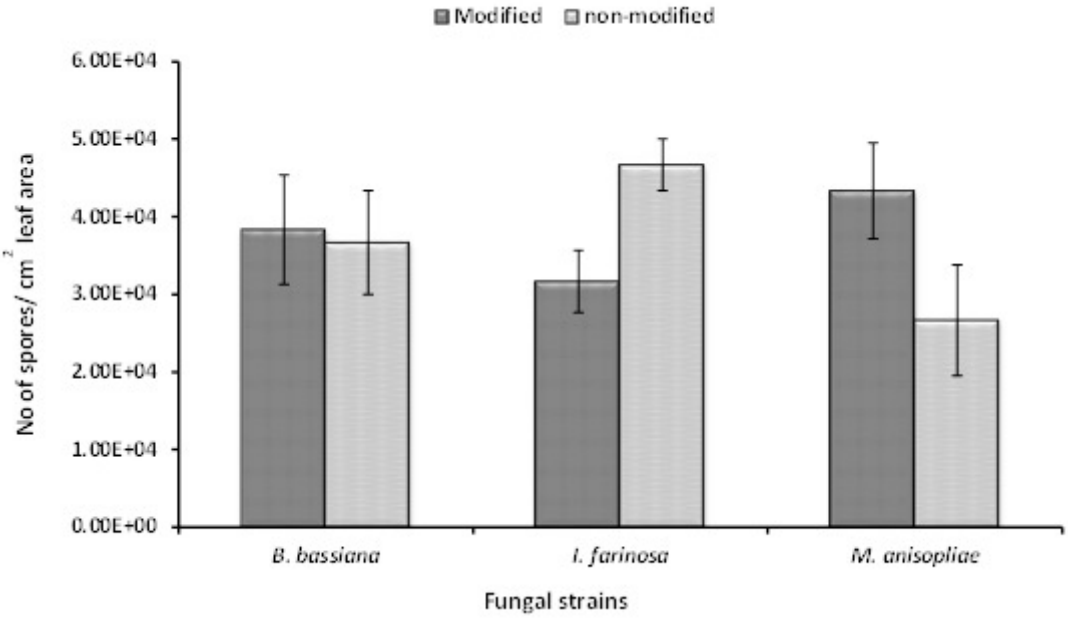

Figure 6. Spore deposit cm^{-2} of cover-slip positioned at abaxial part of tomato leaves. The bars are standard error s of the means.

of the insects into the field, the population was clustered and more than half (55%) of the total sampled leaves had 0-5 insects per leaf. Only 0.5% of the sampled leaves had between 66-70 and 71-75 adult insects, respectively. After 21 days of post-release, the insect population was more evenly distributed; 17% of the sampled leaves had 0-5 insects per leaf while 21% had 6-10 insects per leaf. Only a small percentage of the leaf samples had very high insect densities. This pattern of insect density was peculiar to broad leaves which were close to the ground (1st to 2nd nodes).

Conidia deposit per unit leaf area. There was a significant deposition pattern of conidia between fields sprayed with modified and unmodified conidia of *B. bassiana* ($P<0.05$). For *I. farinosa*, the average modified conidia deposit was significantly lower than observed in the plots sprayed with unmodified conidia. In contrast, the average conidia deposit was significantly higher in areas where modified *M. anisopliae* conidia was applied compared to areas sprayed with unmodified conidia.

Field temperature and relative humidity. The daily temperature and relative humidity for 14 days after treatment during the first trial (Figs. 7a and b), and the second trial (Figs. 8a and b) showed marked variations. For the first trial, 3 hourly variations were recorded between 6 am in the morning and 12 midnight for temperature and relative humidity. It was observed that significant fluctuations only existed between the early morning and the mid-day or the mid-day and the mid-night temperatures and relative humidity. Therefore, in the second trial only 6 hourly temperature and humidity regimes were recorded. The minimum and the maximum temperature recorded during the first trials, respectively, were 16.1 and 46 °C; while the mean temperature was 30.4 °C. Mean relative humidity was 44.8%, while minimum and maximum relative humidity were 20 and 86%, respectively. During the second trial, a lower temperature was recorded compared with the first trial (26.21 *vs* 30.4 °C); and the corresponding mean relative humidity was higher (58.9 *vs* 45%). The minimum and the maximum temperature during the second trial were 16.9 and 43.6 °C, respectively; while the corresponding relative humidity were 20.5 and 86.3%. Highest relative humidity and lowest temperature were also recorded during the morning and mid-night periods. Humidity was low in the mid-day periods when temperature records were at the peak. A localised wide variation in temperature (sometimes up to 5°C) at different plant canopy areas on the plots was observed.

Status of *B. tabaci* density after treatment. Figures 9 and 10, respectively, show the trends of decline in adult *B. tabaci* density in the 1st and the 2nd trials, after separate application of the biocontrol agents and Chlorpyrifos. Under field conditions presented above for the 1st and the 2nd trials, mean densities (average number of insects per leaf) in plots treated either with the biocontrol agent or Chlorpyrifos declined significantly ($P<0.05$) relative to the density before treatment. There was no contrasting difference in the trends of decline in pest density in the first and the second trials; therefore, the mean decline in density each sampling day relative to the density before treatment was pooled and calculated for the 2 trials (Table 1). The mean density of adult *B. tabaci* in the control was always the highest each sampling day during the 1st and the 2nd trials.

The unmodified and modified *B. bassiana* conidia caused 75-96% and 89-98% decline in the mean pest density between 2-14 days, respectively (Table 1). The mean decline in pest density caused by Chlorpyrifos treatment during the same period was 78-97%. In the control, a mean decline in density, 9 and 6%, respectively was observed on the 2nd and 4th day after the control treatment. Thereafter, an upsurge in pest density approximately 24 and 31% at the 7th and the 14th day sampling occurred.

There was a significant decline ($P<0.05$) in pest density for each sampling day after treatment, relative to the pest density before treatment for all the biocontrol agents used and Chlorpyrifos. However, there was no significant difference ($P>0.05$) between the level of pest decline caused by the modified conidia, unmodified conidia and chlorpyrifos.

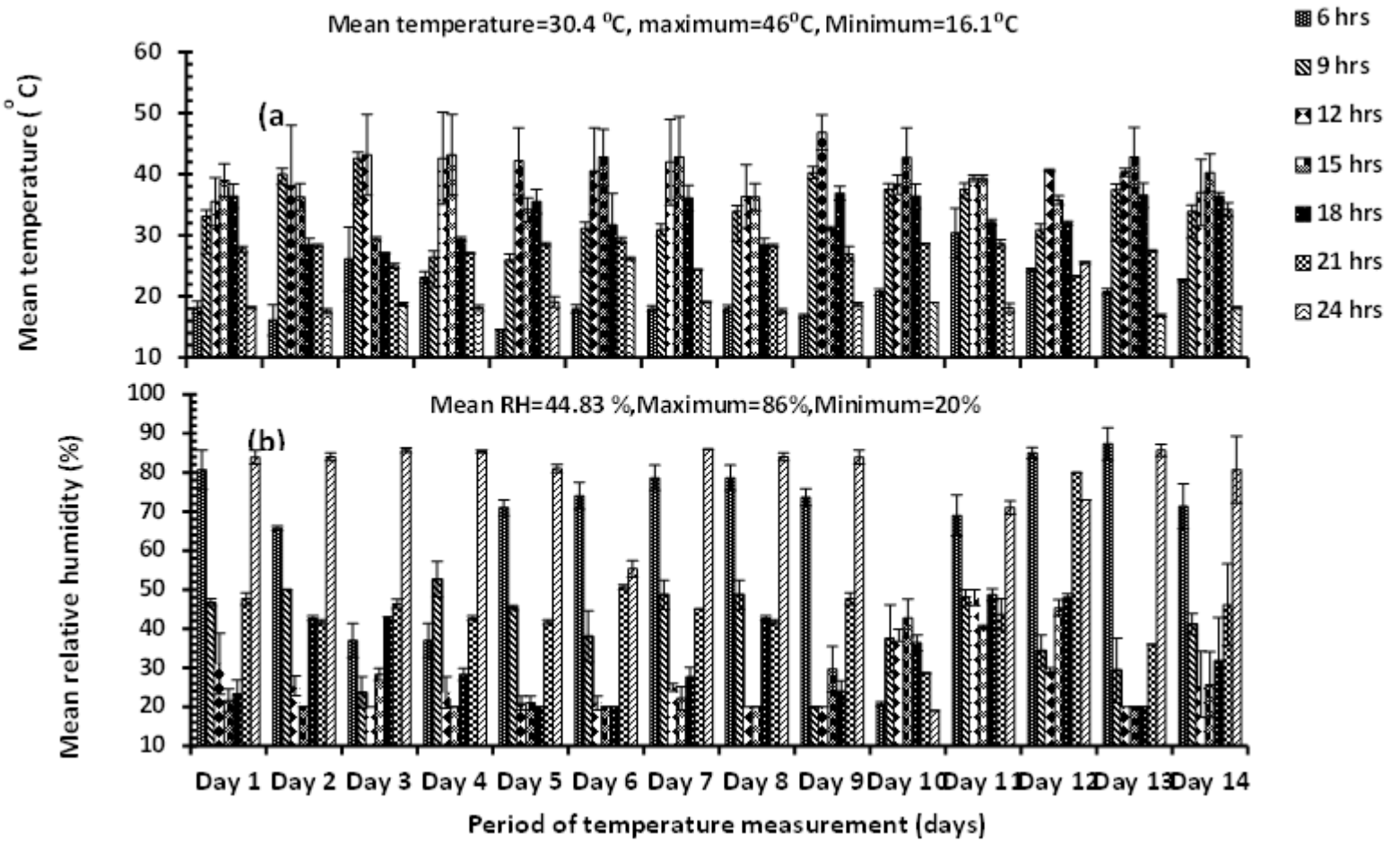

Figure 7. First trial records of 3-hourly (a) field temperature, and (b) relative humidity fluctuations for 14 days after the application of conidia formulations. The bars represent the standard errors of the means.

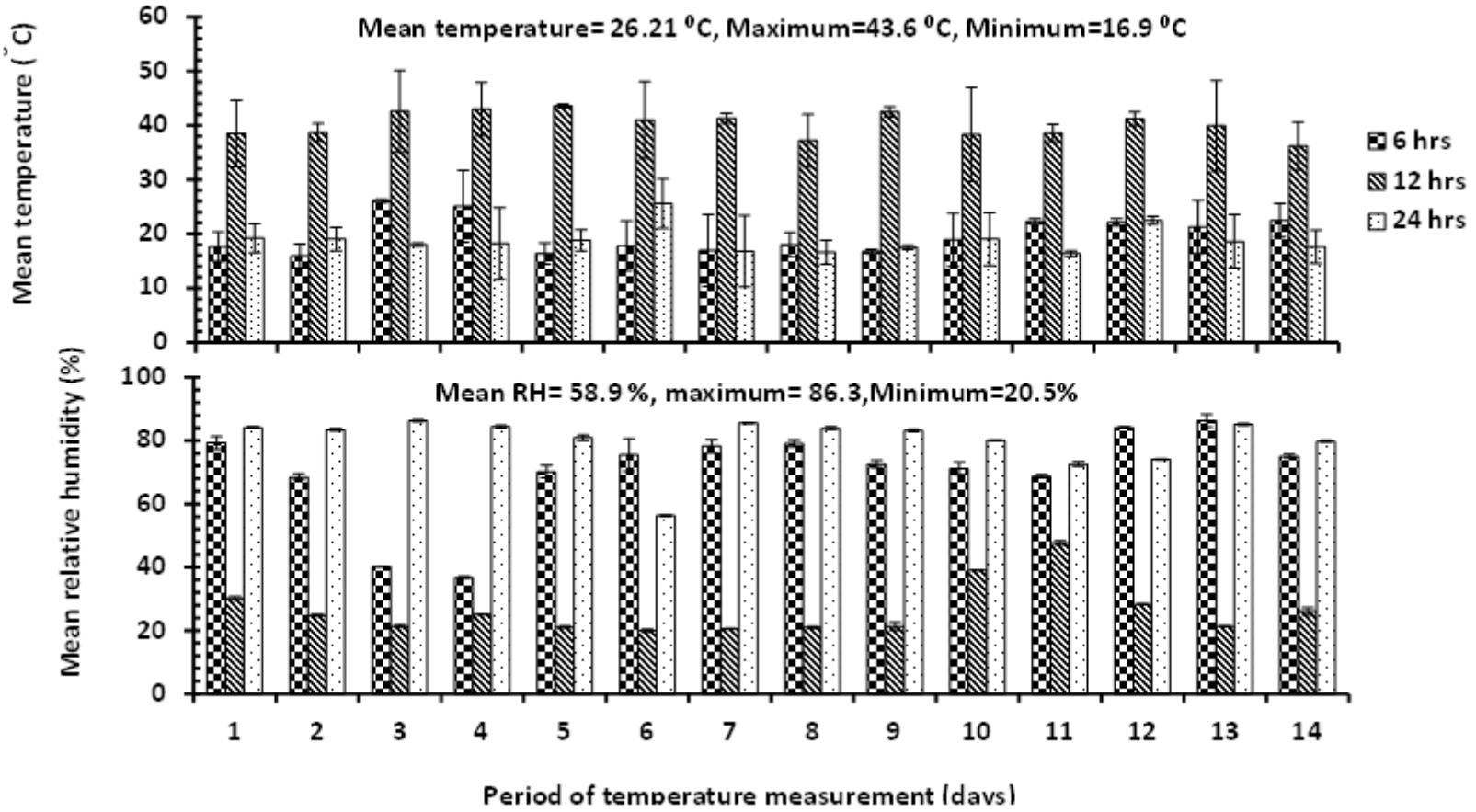

Figure 8. Second trial record of 6-hourly (a) field temperature and (b) relative humidity fluctuations for 14 days after the application of conidia formulations. The bars represent the standard errors of the means.

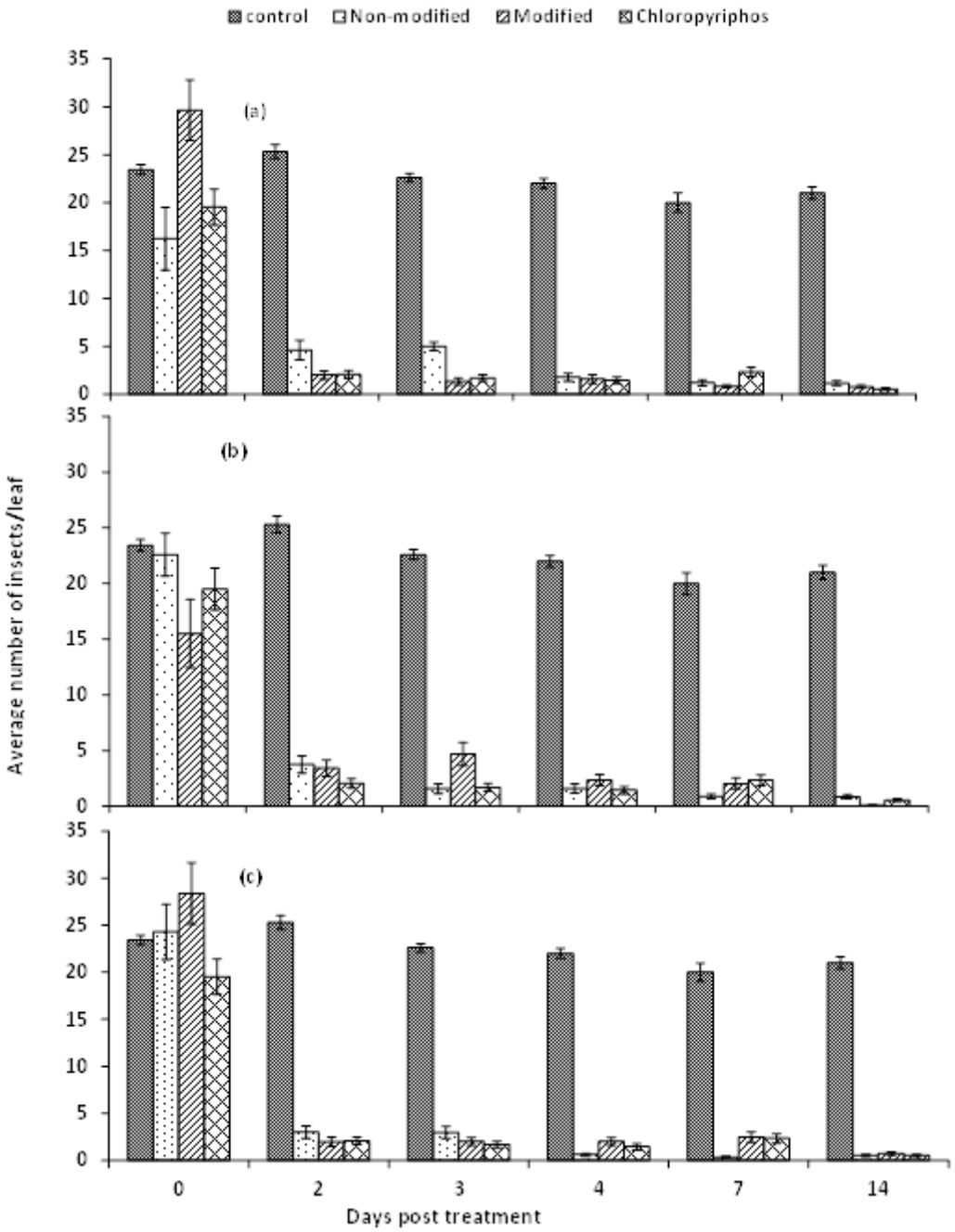

Figure 9. First trial: Trends in *B. tabaci* adult populations on tomato plots separately treated with formulation of modified and unmodified conidia of (a) *B. bassiana,* (b) *I. farinosa* and (c) *M. anisopliae* and Chlorpyrifos. Bars are standard errors of the means where shown.

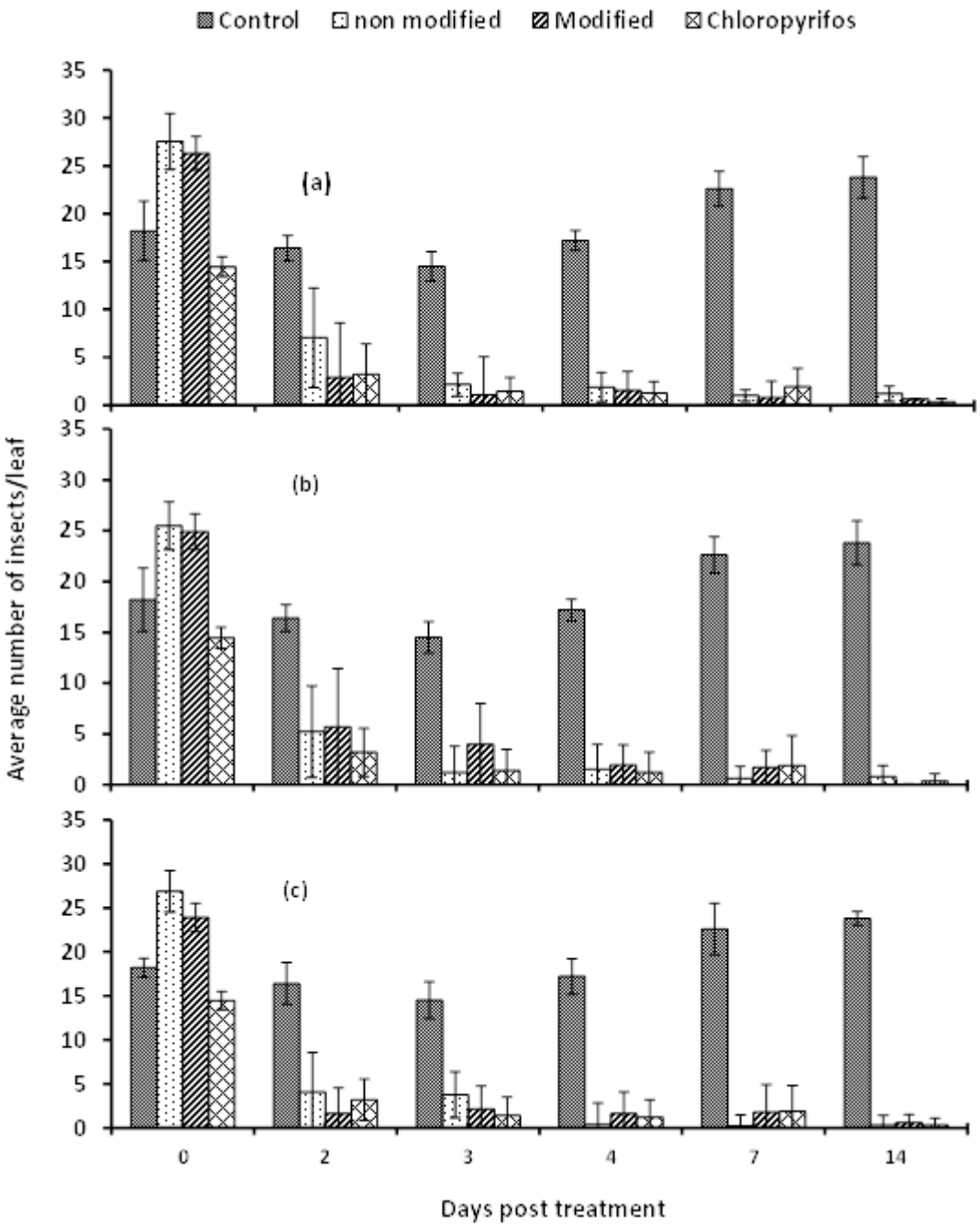

Figure 10. Second trial: Trends in *B. tabaci* adult populations on tomato plots separately treated with formulation of modified and unmodified conidia of (a) *B. bassiana,* (b) *I. farinosa* and (c) *M. anisopliae* and Chlorpyrifos. Bars are standard error of the means where shown.

TABLE 1. Reduction in average density of adult *B. tabaci* on artificially infested tomato after treatment with modified and unmodified conidia of entomopathogenic fungi and Chlorpyrifos.

Fungal strains/other treatments	Formulations	Initial mean population density (Adult *B. tabaci* leaf^{-1}) before treatment	Days post treatment			
			2	4	7	14
			Percentages of the initial population killed after treatment			
B. bassiana	Unmodified	16	74.45	93.26	96.37	95.75
	Modified	29	89.14	94.14	97.07	97.61
I. farinosa	Unmodified	22	79.46	93.94	97.64	96.86
	Modified	15	77.04	92.08	93.11	99.77
M. anisopliae	Unmodified	24	84.81	98.51	99.15	98.72
	Modified	28	92.98	93.19	92.59	97.61
Chemical pesticide	Chlorpyrifos	20	77.87	91.5	86.76	97.63
Water +1% peanut oil+0.05% Tween 80	Control	23	8.89	5.49	*24.18	*30.77

Control consisted of spray with RO water containing 0.05% Tween 80 and 1% vegetable oil. Asterik * in the control column shows increase in population density rather than reduction

DISCUSSION

Visual observations showed that many factors such as leaf geometry, orientation position and size as well as shade (plant canopy) influenced the distribution patterns and density of *B. tabaci* per plant. Deformed (scrolled) leaves for example, were found to harbour more of eggs and other developmental stages than it supported aggregation of adult insects. Wide or large leaves supported large numbers of adult insects, sometimes up to 75 adults per leaf (Fig. 5). More adults were found on the lower vegetal stratum, while dense canopy areas generally supported relatively larger proportions of all life stages. Young leaves at the plant tips supported highest egg density (data not shown).

Hou *et al*. (2007) similarly observed that position of leaves affect within-plant distribution of different life stages of *B. tabaci* on potted cucumber in the green house. Visual observations of adult insects on leaf surface is known to provide the most accurate information and a practical method suitable for estimating white fly population densities (Gusmao *et al*., 2005; Bacci *et al*., 2008).

The results of field application of the biocontrol agents (Table 1) showed that conidia formulation of *B. bassiana, I. farinosa* and *M. anisopliae* were effective for the management of *B. tabaci* on irrigated tomato under field conditions. However, no previous information is available on comparative study of modified and unmodified conidia of entomopathogenic fungi for the control of *B. tabaci* in the field. The results of this study are promising; it may be possible in the future to integrate the use of fungal biocontrol agents with novel pest control methods within the mainstream of African farming framework.

During the first and the second trials, between 75 to 93% reductions in the mean adult *B. tabaci* density was achieved after 48 hours of application of the biocontrol agents; and there was no significant upsurge in the pest density each sampling day for the entire duration of the study. A higher level of success (85-98%) was achieved elsewhere by Wraight *et al*. (2000), with entomopathogenic conidia formulation, but this was after 5 repeated sprays at 4-7 days intervals and with a higher concentration of inoculum. In the current study, incursion of flying adults into the field was restricted before treatment, and this may have contributed to the high level of success achieved with a single spray.

It may be suggested that a combination of physical barriers with biocontrol agents could enhance the level of success. In the control, there was about 6 to 9% reduction in the mean density of the adult insects at 2-4 days. Probably, some adult insects were drowned in the spraying process by the control treatment. Overall, the level of success of the biocontrol agents was comparable to low level Chlorpyrifos.

Poprawski *et al*. (2000) in contrast, reported a significant inability of *I. fumosorosea* and *B. bassiana* to control the 3rd instar of *Trialeuroides vaporariorum* reared on tomato, compared to nymphs reared on cucumber and hypothesized that a glycoalkaloid, tomatine may be responsible for antimicrobiosis on tomato leaves. The host plant-biocontrol agents interaction is an important factor in biological control, but such interaction was not observed in this study.

The mean temperature during the first and the second trials (Figs. 7a and 8a) were within the temperature range required for growth and infection by the 3 strains used in this study (Borisade and Magan, 2014). On the other hand, the mean relative humidity levels during the period (44 and 58.9%), respectively, were far below the requirement to achieve an effective control in the laboratory (Borisade and Magan, 2015). Comprehensive information on temperature and a_w requirements for the growth and sporulation of 6 strains of entomopathogenic *B. bassiana*, 7 strains of *I. farinosa*, 5 strains of *M. anisopliae* and a strain of *I. fumosorosea* which included the strains used in the current field trial have been previously reported (Borisade and Magan, 2014). However, the temperature and relative humidity records showed wide variations between morning through the mid-day and night, both in the 1st and 2nd trials. For example, in the first and the second trials, the maximum temperature were 46 and 43.6 °C, respectively; while the temperature dropped to 16.1 and 16.9 respectively, at midnight. The maximum relative humidity attained in either of the trials was 86.3%, which was lower than

needed to support growth under laboratory studies. It is well known that the performance of biocontrol agents is influenced by atmospheric temperature and relative humidity (Roberts and St. Leger, 2004; Sharififard *et al*., 2012), they were not limiting conditions in this study.

It can be suggested that the plant canopy microclimate and the virulence of the inoculum to the target pest were more important than the prevailing atmospheric temperature and relative humidity. This is consistent with an earlier observation by Shi *et al*. (2008) whereby *B. bassiana* and *M. anisopliae* were used for the control of cotton spider mites.

There was a wide variation in the records of the temperature meters placed at different canopy areas. It can be further suggested that the success of the biocontrol agents also depended on their ability to exploit favourable periods of optimum humidity and temperature within the microclimate or the macroclimate for growth and infection. In addition to this, the ability of the biocontrol agents to withstand extreme temperature and moisture stress, without a significant loss of germination capabilities, contributed to their biocontrol potentials and persistence in the field. A prolonged low relative humidity and high temperature may be more important to secondary spread than a deterrence to initial control, particularly when emulsifiable liquid formulations are used.

The modified conidia of each of the 3 strains had significantly ($P<0.05$) higher germination only at the water activity level which corresponded to the media on which it was cultured (0.98 a_w). The expectation was that modified conidia would germinate faster at the 3 a_w levels tested.

A conidium which failed to germinate within 24 hours does not imply a dead one (Morley-Davies *et al*., 1996). Heat stress has been shown to adversely affect germination rates (Faria *et al*., 2007) and a longer incubation period may be required for germination to take place. Tolerance to heat stress is a critical determinant of virulence and persistence of fungal conidia in the field, particularly under tropical climate where high temperatures are often encountered. It was interesting that the maximum temperature encountered in the field was within the range used in the preliminary studies on the 3 strains prior to the field work (Fig. 3).

In this study, it was relatively difficult to describe the time to death of the sedentary nymphs. Discolouration of the nymphs was the only index which was used to assess mortality. It is worthy to note that toxins also play an important role in insect mortality and it may be possible that some nymphs died earlier than the time a visible discolouration was noticed. Entomotoxic effects of metabolites derived from entomopathogenic *M. anisopliae* (Sree *et al.,* 2008) and *B. bassiana* (Quesada-Moraga *et al.*, 2006; Ortiz-Urquiza *et al*., 2010) have been reported in many insects.

Conidia delivery method is known to affect inoculum deposit at different vegetal stratum (Bateman and Chapple, 2001). The conidia deposit cm^{-2} of leaf area varied significantly on different plots, but the variation did not significantly affect field performance. Shi *et al*. (2008) reported no significant difference in the field performance of either *M. anisopliae* or *B. bassiana,* when applied at different concentrations (1.5×10^{13} and 1.05×10^{13}) to citrus red mites (*Panonychus citri*). The study suggested that field efficacies were less associated with LC_{50} values obtained in the laboratory than the potential adaptation of the inoculum to abiotic interactions.

Selection of virulent strains which are naturally tolerant to abiotic stress factors should be a priority from ecological standpoint. Thereafter, such tolerant strains can be improved and formulated to achieve better level of control in environments which are less favourable to biocontrol with fungi.

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