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Research Article

Biological control of cashew powdery mildew using *Ampelomyces quisqualis* Ces

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ABSTRACT: The evaluation of the possibility of exploiting *Ampelomyces quisqualis*, mycoparasitic fungi for the biocontrol of cashew powdery mildew (*Oidium anacardii*) was studied. An in vitro biological control test on detached leaves of cashew was used and a detailed microscopic analysis of the interactions between mycoparasite and *O. anacardii* conducted. Effect of mycoparasite on disease severity and incidence was discussed. *Ampelomyces quisqualis* was confirmed as a mycoparasite of *O. anacardii*. When grown near the pathogen, *A. quisqualis* was seen entwining around the pathogen mycelium. It was stimulated to produce branches that grew directly to the mycelium of pathogen *Ampelomyces quisqualis* firm attachment on the pathogen conidia resulted in the penetration and successful growth. Some of the impregnated *O. anacardii* conidia were found dead. Koch's postulates were satisfied to establish the mycoparasitism of *A. quisqualis*.

KEY WORDS: *Oidium anacardii,* cashew, *Ampelomyces quisqualis, biological control*

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INTRODUCTION

Cashew (*Anacardium occidentale* Linn.) is an economically important cash crop across Tanzania producing areas which include Mtwara, Ruvuma, Lindi, Coast and Tanga regions. However, due to the emergence of diseases, the production is hampered. Powdery mildew (*Oidium. anacardii*) is the most important disease in cashew causing nut yield losses of upto 70% (ACRR, 2006). Control of powdery mildew of cashew is mainly by chemical application using sulphur dust (Sijaona and Mansfield, 2001). There is widespread use of sulphur and systemic fungicides to control powdery mildew in Tanzania. Indiscriminate use of these chemicals has often resulted in adverse environmental effects, development of pest resistance and negative effects on human health (Slusarenko *et al*., 2008). Chemical applications bear the risk of acidifying soils and this threatens the productivity of cashew nut (Ngatunga and Dondeyne, 2001). Application of fungicides to control powdery mildew fortnightly, as practiced by some farmers, is overuse of fungicides and could lead to high residue levels in the harvested produce. Many pesticides are not easily degradable; they persist in soil, leach to groundwater and surface water and contaminate the environment. Biological control agents have received most of the attention because of their versatile modes of action to protect plants and their potential to be included in integrated management programmes (Romero *et al.,* 2007). Pycnidial fungi belonging to the genus *Ampelomyces* have been documented to be intracellular mycoparasites of powdery mildew (Szentiványi *et al*, 2003). Genus *Ampelomyces* belong to the class of coelomycetes that are widespread, thermophilic and adapted to various climatic conditions (Sucharzewska *et al.,* 2011). *Ampelomyces* strains are hyperparasites of several powdery mildews. Although the use *Ampelomyces* isolates as biological control agents of powdery mildews has been tried on various crops, little is known about their effect on cashew powdery in the field. The main objectives were: (i) to collect plant materials known to be infected with powdery mildew during the previous season; (ii) to identify pycnidia and other structures of *Ampelomyces* on these plant materials after inoculation; and (iii) to test their viability and mycoparasitic activity against cashew powdery mildew. Biocontrol agents could be tried as safe potential alternative to chemical control of plant pathogens. In the search for new eco-friendly and non-toxic agrochemicals to control powdery mildew, *A. quisqualis* strains were tested as potential biocontrol against powdery mildew (*Oidium anacardii* Noack) in cashew. The current investigation was planned to study the ability of *A. quisqualis* mycoparasitic fungus to control powdery mildew disease of cashew plants under in vitro and field conditions.

MATERIALS AND METHODS

The study was conducted in 2011-2012 at Naliendele Agricultural Research Laboratories, Mtwara (040° 09' 15.61" E 10° 22' 40.98" S, 141 meters above sea level) from Jan 2011 until March 2012. The rainy season of November/December to April/May is single peaked, the peak being reached in January but occasionally in February or March. Mtwara district rains vary from 935 mm to 116 mm in the hills and the plateau. Likewise temperatures vary from 27°C as the highest monthly mean at Mtwara on the coast in December to 23°C in July. Relative humidity goes from 87% in March to 79% in October in Mtwara. Temperatures and humidity are lower inland. In total eight powdery mildew populations were collected in main growing areas in Tanzania. Collection sites were located in Mtwara. Leaf samples were taken in fungicide free plots of a susceptible cultivar during the occurrence of primary powdery mildew symptoms. Maintenance of the obligate biotrophic fungus was carried out on the highly susceptible cultivar cashew clone AC 10. The fungal inoculum used in the experiment was obtained from plants naturally infected with powdery mildew in a field of selected cashew growing areas. Infected leaf materials of susceptible cashew types were established. Subcultures of *Ampelomyces quisqualis strains* (DSM 2222) were obtained from Hungarian Academy of Sciences, Hungary. The strains were cultured on modified Czapek-Dox medium supplemented with 2% malt for mass production. The recipe of the modified Czapek-Dox medium was as follows: NaNO3 (1.5g), KH2PO4 (0.5g), KCl (0.25g), MgSO4 (0.25g) and malt (10g) in 500ml of distilled water.

Viability tests

Conidial suspension of *A. quisqualis* adjusted to 10^6 conidia per ml using Neubar hemacytometer was sprayed on 1.5% water agar medium and incubated for 24 hat 27°C and then checked the cells under a light microscope (approx. 400x). After 24 h incubation at room temperature, percent germination was examined under the microscope.

In Vitro mycoparasitic activity of *Ampelomyces quisqualis* on *Oidium anacardii*

Cashew leaves with natural mildew infection were selected 24 h before each inoculation date and shaken to dislodge old spores and encourage production of fresh spore overnight. Using camel hair brush method (Shomari, 1996) the inoculums was transferred from the source surface into the sterilized slides put in 9cm petridishes lined with moistened sterilized filter paper with sterilized tap water. Twenty-eight days old culture of *A. quisqualis* spore suspension (10^6 spores/ml) was prepared in sterilized tap water. A drop of *A. quisqualis* spore suspension was placed on early inoculated slides (25x75 mm in size) with powdery mildew. Dual cultures were incubated at 27± 2°C. Observations on spore germination were recorded at 11 d after incubation for sporulation activity. Observations were also made on germination. Light microscopy was used to examine penetration, internal growth and sporulation of *Ampelomyces* in powdery mildew mycelia covering the cashew leaves. The experiments were repeated twice.

Mycoparasitism of *Ampelomyces quisqualis* DSM 2222 against cashew powdery mildew in controlled conditions

Detached leaf assay

Leaves from the 2nd and 3rd nodes of young healthy 4 cashew clones were collected and superficially sterilized by immersion in 70% ethanol for 1min (Pruvostand Luisetti, 1991). Leaf discs were excised with a 20mm diameter cork borer which was surface sterilized in a 3.85% m/v sodium hypochlorite solution for 1min and was rinsed in sterile distilled water. The discs were placed on sterile Whatman no. 1 filter paper saturated with sorbitol (aqueous concentration 2%w/v) (Nathaniels.1996). A completely randomized block design with 6 treatments in 3 replicates was used. The powdery mildew pathogen was inoculated on the 24 h pre-treated discs by camel hair brush method. Each replicate consisted of 3 Petri-dishes with 3 leaf discs in each. The treatment consisted of *Ampelomyces quisqualis* suspension adjusted to 10^6 conidia per ml. Pathogenecity was determined by estimating the percent collapse of spores by recording the number of erect spores out of approx 100 spores at the point of inoculation and sporulation by counting the number of *A. quisqualis* pycnidia per cm^2 of powdery mildew colony after 12 d. The upper leaf surfaces of cashew seedlings and the lower surfaces of cashew leaves were examined. Leaf bits containing abundant pycnidia of the *A. quisqualis* were cut into 4mm x 4mm leaf sections, placed directly on tap water agar and the incubated at room temperature. The fungus development was observed. The experiments were repeated twice.

Screening of *Ampelomyces quisqualis* on cashew powdery mildew seedlings

An isolate of *A. quisqualis* was screened for pathogenecity and ability to sporulate on *O. anarcadii*. Seeds of the cashew were supplied by ARI, Naliendele. 100 seeds were sown in soil-sand mixture enriched with N.P.K fertilizer on 2L plastic pots. The pots were maintained in a green house and watered daily. After 3 weeks, grafting was carried out on the seedlings using for four cashew clones selected. Each clone had twenty five grafted seedlings. Vegetatively-propagated cashew clones ensured more uniform agronomic characteristics. Four months old seedlings

were used for experiments. Leaves were inoculated with *O. anarcadii* obtained from cashew infected trees in Mtwara, Tanzania, by transferring conidia by using camel hair brush method. The three treatments included: (i) plants treated with tap water (ii) *A. quisqualis* (iii) Fungicide Mupafidan (triadimenol 250 Emulsifiable Concentrate as positive control). After 6 d, powdery mildew colonies were inoculated with *A. quisqualis,* held humidity chambers made by placing polythene wrap over the cups for 24h, and then removed. Tap water was sprayed onto cashew seedlings immediately before inoculation of the mildew colonies with *A. quisqualis*. The conidial suspensions were enumerated with a hemacytometer, and were adjusted to 10^6 conidia per ml, thereafter sprayed on the young leaves of each of nine selected cashew clone seedlings. Control plants in each clone were inoculated with tap water. All treatments were replicated on 10 seedlings and the experiment was repeated. Disease severity was scored 5 d, 10 d and 15 d after powdery mildew inoculation using 0-6 disease severity scale (Nathaniels.1996). Disease incidence and severity on leaves was assessed as number of infected leaves per plant (Yun *et al.,* 2011).

***Percent Disease Incidence (PDI) was Calculated using the Formula*:**

$$\text{PDI} = \frac{\text{Number of diseased leaves on each plant}}{\text{Total number of leaves of each plant}} \times 100$$

Percent Disease Reduction (PDR) was Calculated Using the Formula

$$\text{PDR} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Mycoparasitic tests using leaf discs infected with powdery mildew

To test the capacity of penetration, internal growth and intracellular sporulation of the structures of *Ampelomyces*, they were transferred from the plant materials to leaf discs covered by young and sporulating powdery mildew colonies. Leaf discs were produced by cutting out discs of 20 mm diameter from cashew leaves infected with *A. quisqualis.* Leaf segments were kept in plates containing moist filter paper. Two or three cashew leaf discs infected with powdery mildew were inoculated with pycnidia or hyphae of *Ampelomyces*. Non-inoculated leaf segments served as controls and were sprayed with sterile water. The plates were covered for 48h to ensure 100% relative humidity in the plates, and kept in an incubator at 27°C with 12 h daily illumination period. After 48 h, plates were opened and kept in the incubator for 12 more days before the leaf segments were examined under a light microscope for the presence of intracellular pycnidia characteristics of *Ampelomyces* in the mildew mycelia. To fulfill Koch's postulates, the mycoparasites were re-isolated from the inoculated cashew leaves.

RESULTS AND DISCUSSION

The fungal pathogens involved in powdery midew disease of cashew were isolated from leaves collected from different regions of Tanzania and were identified. The pathogenecity was done and the Koch's postulates were fulfilled.

In Vitro* mycoparasitic activity of *Ampelomyces quisqualis* on *Oidium anacardii

Microscopic examination of cashew diseased tissue revealed hyaline, cylindrical-to-slightly doliform, single-celled conidia borne in chains. The pathogen was subsequently identified as *Oidium anacardii* Noack on the basis of morphology. Hyphae of the *Ampelomyces* were hyaline and septate; they were present within the hyphae, conidiophores, and conidia of infected mildews. Pycnidia were light brown in transmitted light and varied in shape from sub-globose to pyriform (Fig 1). Pycnidia contained one-celled, hyaline, guttulate conidia which were ovoid, clavate, cylindrical, or curved. Conidia are hyaline or pale brown, smooth, without septa, with rounded, straight or slightly curved ends. *Ampelomyces quisqualis* pear-shaped pycnidia develop in the *O. anarcadii* conidiophores. *A. quisqualis* was observed on the mycelia of *O. anarcadii*. The percentage germination rate of *A. quisqualis* was over 90%. When growing near *O. anarcadii* hyphae, *A. quisqualis* was stimulated to produce branches that were directed towards the *O. anarcadii* mycelium (Fig 2). Water soluble substance from cashew powdery mildew conidia might be the one responsible in stimulating the germination of *A. quisqualis. Ampelomyces quisqualis* might be directly invading and destroying the powdery mildew cytoplasm thus reducing its growth.

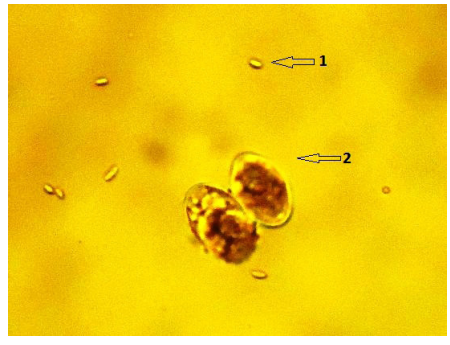

Fig. 1. *Oidium anacardii* (arrow 2) and *Ampelomyces quisqualis* (arrow 1) conidia.

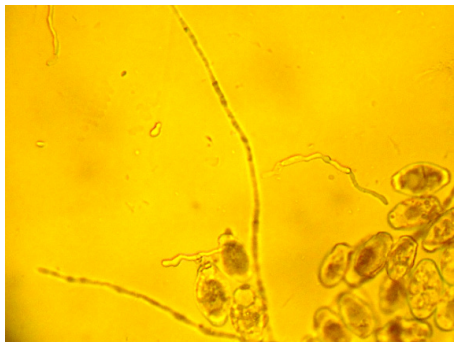

Fig. 2. ***Ampelomyces quisqualis*** **germinating spore was seen recognizing the presence of** ***Oidium anacardii*** **conidia, initiating attachment.**

Penetration of the host and internal growth of hyphae was observed (Fig 3). Overlap of the hyphae of *A. quisqualis* began to form after 5 days entwining around the mycelium was a characteristic response of *A. quisqualis* to the pathogen after penetration (Fig 4). The pathogen appeared to have stopped growing after penetration. *O. anarcadii* is characterized by short like chains of conidia. Directed growth of germ tubes of *Ampelomyces* towards *O. anarcadii* hyphae was observed. Parasitized powdery hyphae continued their radial growth but it seemed like sporulation stopped soon after *A. quisqualis* penetrated their mycelia. Hyphae of *A. quisqualis* penetrated the cashew powdery mildews, continued with growth internally and produced their pcynidia in the cells of the hyphae (Fig 5). The presence of the active mycelium could have caused the degradation of hyphal walls of cashew powdery mildew fungus. This could be attributed to rapid degeneration of cell contents of *O. anacardii* conidia which were found impregnated due to the penetration of *A. quisqualis* spores, thus causing the death of pathogen conidia (Fig 6).

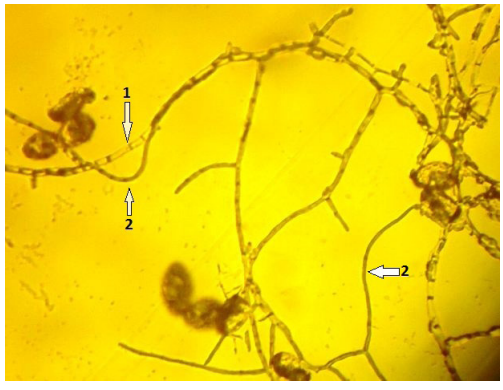

Fig. 3. **Successful penetration on** ***Oidium anarcadii*** **conidium (arrow 1), resulted in the growth of** ***Ampelomyces quisqualis*** **hyphae (arrow 2).**

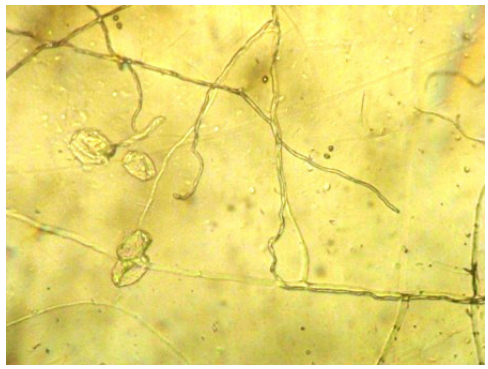

Fig. 4. **Entwining of long-chain of** ***Oidium anacardii*** **by** ***Ampelomyces quisqualis*** **hyphae.**

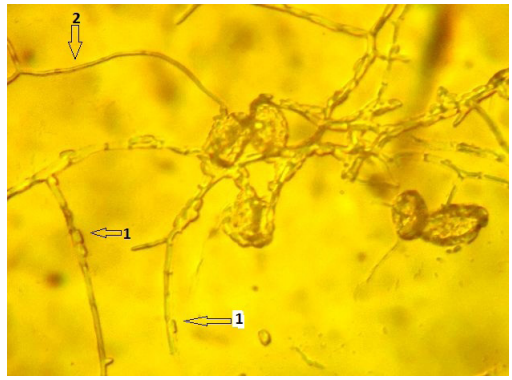

Fig. 5. **Attachment of dislodged conidia of** ***Ampelomyces quisqualis*** **on** ***Oidium anacardii.***

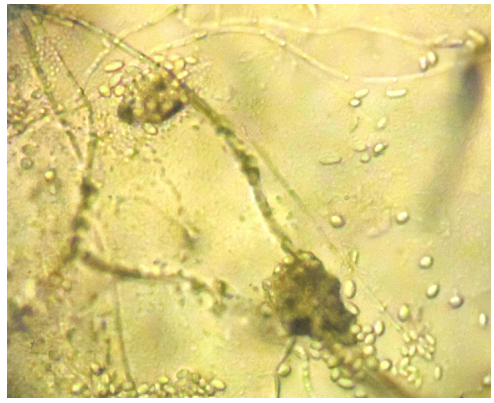

Fig. 6. **Impregnated spore of** ***Oidium anacardii*** **observed enlarging thus causing it to burst and releasing** ***Ampelomyces quisqualis*** **conidia.**

Mycoparasitic tests using leaf discs infected with powdery mildew

Microscopic examination of the leaves revealed the presence of *A. quisqualis* black pycnidia emerging through the Powdery mildew pathogen on the leaf surface (Fig 7 and 8). The fungus started developing rapidly into sparse

colonies within four days. Pear-shaped brown pycnidia appeared. The spores of *Ampelomyces* were produced in pycnidia that developed inside powdery mildew conidiophores and fruiting bodies (Fig 9). These grow superficially as white or creamy white cover on host surface and draw nutrition through haustoria. Chronic infections caused by powdery mildews are generally associated with stunting and distortion of leaves, surface necrosis of infected tissue, a general decline in host growth, deformation of fruit, yellowing or chlororsis of leaves, and premature leaf fall. Cultivated plants, viz. apple, mango, peach, peas, potato, rose, rubber, tobacco, tomato, and wheat are attacked by powdery mildews. Powdery mildew mycelia appeared as grayish, powdery masses on infected leaves. Older patches of powdery infection turned yellow. Powdery mildew fruiting bodies were observed as brown specks. Infected leaves curl, drop and in the field there is abortion of fruits. There were significant differences in powdery mildew conidiophore collapse (%) among the clones after inoculating with *A. quisqualis* on detached leaves ($P<0.05$). The conidiophores collapse percentage was high in all clones tested ranging from AC4 (74%), AC10 (76%), AZA2 (87%) and AZA17 (88%) respectively. General the mean conidophore collapse (%) was 81% in all clones. There were significant differences in number of pycnidia per cm^2 on the detached leaves among the clones studied ($P<0.006$). AC4 had the highest number of pycnidia per cm^2 (63%) followed by AZA17 (62%), AC10 (58%) and AZA2 (53%). The average number of pycnidia per cm^2 in all cashew clones was fifty nine.

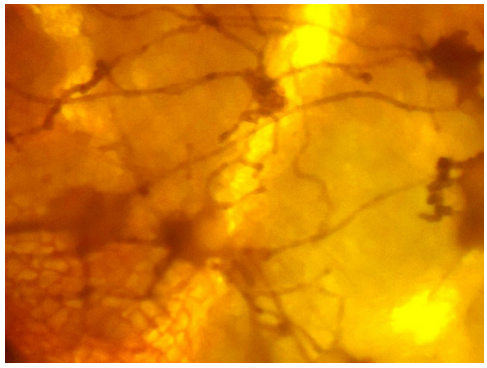

Fig. 7. Powdery mildew growing on a leaf tissue with *Ampelomyces quisqualis* parasitizing.

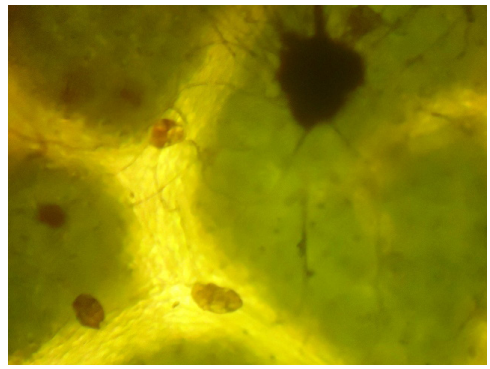

Fig. 8. Mycoparasite conidia extending into *Oidium anacardii spore*.

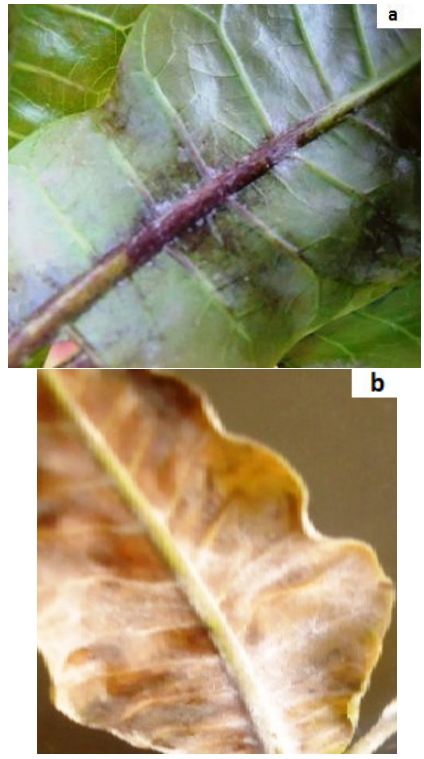

Fig. 9. (a) parasitized colonies of cashew powdery mildew are dull in appearance, flattened off-white to gray in color with reduced spore production (b) brown leaf with natural mildew infection.

Screening of *Ampelomyces quisqualis* on cashew powdery mildew seedlings

Results of the present study indicate the significant difference in the different clones of cashew with respect to powdery mildew infection. The incidence and severity of the disease depends on local agronomical conditions, local practices and season. The artificial inoculation with *O. anarcadii* resulted in high infection levels in all trials (Table 1-4), with mean disease incidence ranging at the end of the trials in the untreated controls (water), from 62.50 -77.08% and 70.83 -81.25% in trial I and II respectively. There were significant differences (P<0.0001) in percent disease incidence among the clones studied in trial I (Table 1).In trial 1, *A. quisqualis* treated cashew seedlings recorded relatively low disease incidence levels ranging from 44.79% to 68.33% in cashew clone AC4 while the untreated control (water) revealed high incidence levels ranging from 62.50% to 77.08%. In trial II (Table 2), *A. quisqualis* treated cashew plants recorded between 31.43% to 52.78% disease incidences as compared to the untreated control that recorded 70.83 -81.25%. Percent disease reduction in the positive control was higher ranging from 27.92 to 32.67% and 49.98 – 68.42% in trials I and II respectively as compared to *A. quisqualis* treatment. Percent disease reduction of 11.35 -30.64% was achieved when *A. quisqualis* treatment was applied fifteen days after inoculation in trial I while in trial II there was 26.47 – 43.59% disease reduction. This showed that *A. quisqualis* probably exerted some mycoparasitic protection action over cashew seedlings when seedlings. Percent disease reduction of *A. quisqualis* treatment though not significant (P>0.05) when compared with the untreated control in cashew clones AC10 and AC4 in trial I at fifteen days after inoculation was numerically lower, thus providing the effectiveness of the bio agent over the untreated control against the fungus. There were no significant differences in percent disease incidence among the clones studied in trial II (P=0.994).

The effectiveness of the different treatment on percent disease severity and percent disease reduction is as shown in Tables 3- 4 and Fig 10-11.Percent disease reduction of *A. quisqualis* treatment in trial 1 ranged from 16.0% in clone AZA2 to 48.2% in cashew clone AZA17 while that of the positive control was from 22.1% in AZA2 to 53.8% in cashew clone AZA17 (Table 3).

Table 1. Percent disease incidence and disease reduction during Trial I

Treatments	AC10	PDR	AC4	PDR	AZA17	PDR	AZA2	PDR
Water	*77.08[a]	-	64.58[a]	-	62.50[a]	-	.64.58[a]	-
A. quisqualis	68.33[a]	[b]11.35	55.21[a]	14.52	45.14[b]	27.78	44.79[b]	30.64
Positive control	43.75[b]	32.50	35.42[b]	32.67	33.33[b]	27.92	31.25[b]	32.58
Mean	64.81		52.60		46.25		46.25	
CV%	27.85		28.67		31.89		36.86	
LSD	19.242		16.534		15.413		18.73	
P-Value	0.0091		0.0089		0.007		0.0098	

*Values followed by different letters within a column are significantly different according to the LSD test (P = 0.05).
PDR= Percent disease reduction
bPercentage of disease reduction achieved by each treatment referred to values of leaf area covered by powdery mildew in untreated controls.

Table 2. Percent disease incidence and disease reduction during Trial II

Treatments	AC10	PDR	AC4	PDR	AZA17	PDR	AZA2	PDR
Water	*72.92[a]	-	81.25[a]	-	70.83[a]	-	72.92[a]	-
A. quisqualis	52.78[b]	[b]27.62	45.83[b]	43.59	52.08[a]	26.47	50.00[b]	31.43
Positive control	16.67[c]	68.42	22.92[c]	49.98	20.83[b]	60.00	20.83[c]	58.34
Mean	48.21		50.00		47.91		47.92	
CV%	29.76		28.62		38.98		27.08	
LSD	16.411		17.61		22.988		15.969	
P-Value	<.0001		<.0001		0.0012		<.0001	

*Values followed by different letters within a column are significantly different according to the LSD test (P = 0.05).
PDR= Percent disease reduction
bPercentage of disease reduction achieved by each treatment referred to values of leaf area covered by powdery mildew in untreated controls.

Table 3. Disease severity (percent of surface infected area) on cashew plants during trial I

Treatments	AC10	PDR	AC4	PDR	AZA17	PDR	AZA2	PDR
Water	86.12[a]	-	62.33[a]	-	49.83[a]	-	79.17[a]	-
A. quisqualis	68.67[ab]	20.2	46.83[ab]	24.9	25.83[b]	48.2	66.5[ab]	16.0
Positive control	56.00[b]	34.9	33.33[b]	46.5	23.00[b]	53.8	61.67[b]	22.1
Mean	70.04		47.50		32.88		69.11	
CV%	25.43		31.26		34.05		19.80	
LSD	20.37		18.27		13.783		16.842	
P-Value	0.0288		0.014		0.0015		0.040	

*Values followed by different letters within a column are significantly different according to the LSD test (P = 0.05). PDR=Percent disease reduction

Table 4. Disease severity (percent of surface infected area) on cashew plants during trial II

Treatments	AC10	PDR	AC4	PDR	AZA17	PDR	AZA2	PDR
Water	82.16[a]	-	81.00[a]	-	83.33[a]	-	78.83[a]	-
A. quisqualis	32.00[b]	61.1	35.00[b]	56.8	28.44[b]	65.9	29.92[b]	63.0
Positive control	18.00[c]	78.1	19.50[c]	75.9	16.33[c]	80.4	18.83[c]	76.1
Mean	40.03		42.62		37.00		39.37	
CV%	13.28		11.91		7.66		17.05	
LSD	5.66		5.56		2.96		7.358	
P-Value	<.0001		<.0001		<.0001		<.0001	

*Values followed by different letters within

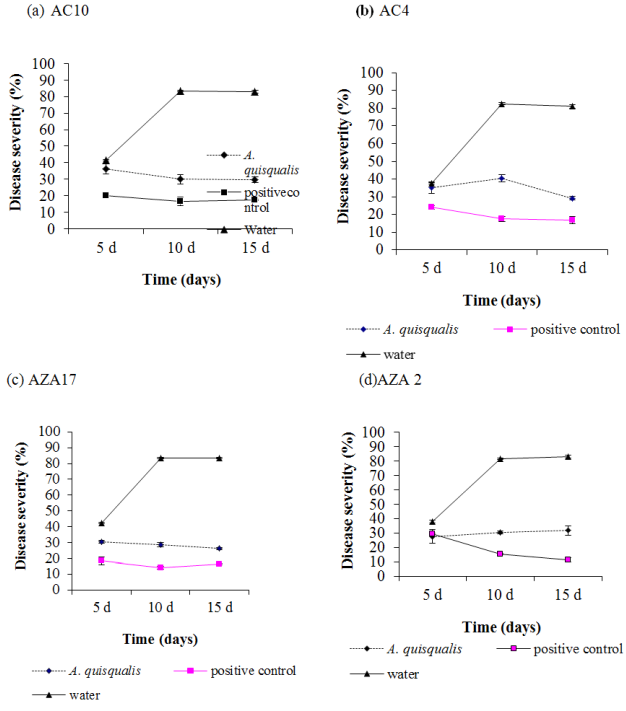

Fig. 10. Disease progress of powdery mildew on cashew and effect of biological control treatment during trial I. Disease severity (percentage of leaf area covered by powdery mildew) measured at 5, 10 and 15 days after inoculation with *Ampelomyces quisqualis.*

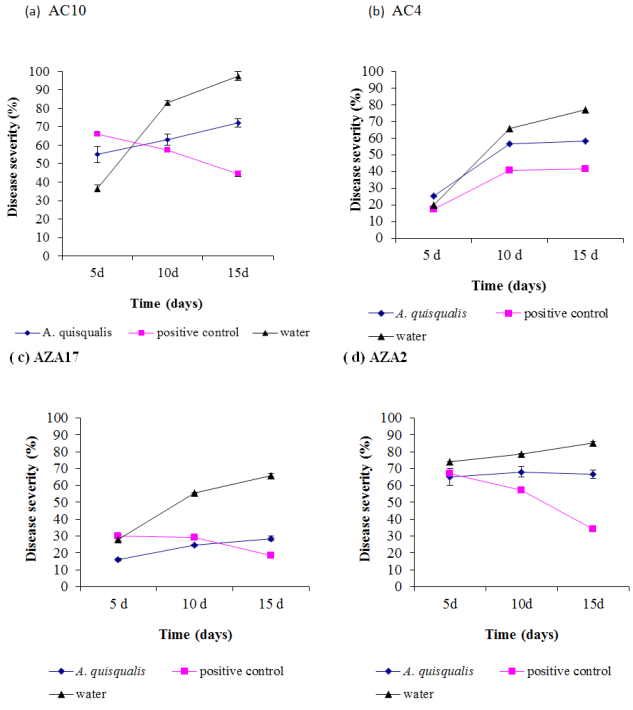

Fig. 11. Disease progress of powdery mildew on cashew and effect of biological control treatment during trial II. Disease severity (percentage of leaf area covered by powdery mildew) measured at 5, 10 and 15 days after inoculation with *Ampelomyces quisqualis.*

Higher disease severity levels in the untreated control during trial 1 (Table 3) were experienced throughout the season whereby AC10 recorded 86.12% followed by AZA2 (79.17%), AC4 (62.33%), and AZA17 (49.83%) respectively. In *A. quisqualis* treatment, cashew clone AC10 revealed the highest disease severity (68.67%) followed by AZA2 (66.5%), AC4 (46.83%) and AZA17 (25.83%) respectively (Table 3).The percent disease reduction in *A. quisqualis* treatment ranged from 56.8% in clone AC4 to 65.8% in cashew clone AZA17 (Table 4). In the positive control, the percent disease reduction was 76.1% - 80.4% (Table 4). To evaluate the suppressive effect of the different treatments on powdery mildew disease progress in field experiments, the severity was recorded (Fig 10 and 11). Inoculation of plants with *A. quisqualis* suspensions adjusted to 10^6 conidia per mL resulted in severe disease symptoms causing severity values of 98% as observed for untreated control fifteen days after inoculation. The experiments were terminated fifteen days after pathogen inoculation, when the infected young tender leaves were mature to withstand the infection. In general *A. quisqualis* and positive control treatments delayed the progress of powdery mildew disease compared with the untreated controls. There were differences on the performance of the treatments depending on the season in which the trials were carried out. The disease severity in trial II was generally higher than in the other trial. Positive control was the most effective control treatment, maintaining powdery disease at low levels during the different trials, independently of the season. High disease severity levels recorded in trial II during March 2012 in all treatments could be due to the high humidity that was experienced in the experimental site (approx 87%). The high humidity is required for adequate germination of *O. anacardii* spores.

The present study was designed to reduce using chemicals in agriculture and find out the most suitable non-chemical method to protect cashew plants against powdery mildew disease. Mycoparasitism has been shown to be an important mechanism of biological control (Brozova, 2004). *A. quisqualis* parasitism on cashew powdery mildew resulted in reduced growth. It infected and produced pycnidia within the cashew powdery mildew hyphae and conidiophores. This has been witnessed in other powdery mildew including grapes and other various crops (Kiss, 2003). *A. quisqualis* mycoparasite can significantly reduce powdery mildew symptoms caused by *O. anacardii*. Spores of *A. quisqualis* penetrated the cashew powdery mildew hyphae and caused the rupture of the *A. quisqualis* hyphal wall. Data obtained showed that *A. quisqualis* significantly reduced disease incidence and severity of cashew powdery mildew. These results might be due to the mycoparasitic activity of *A. quisqualis*. The mycoparasite ramifies throughout the host hyphae, resulting in reduced growth and eventual death of the mildew colony. It infects and forms pycnidia within powdery mildew hyphae, conidiophores, and cleistothecia. This study corroborates with early reports that Pycnidia of *Ampelomyces* attack and is commonly found the cells of the hyphae and conidiophores of powdery mildew fungi worldwide (Falk *et al.,*1995; Kiss *et al.,* 2004).This parasitism reduces growth and may eventually kill the mildew colony. Directed growth of germ-tubes of *Ampelomyces* towards cashew powdery mildew hyphae was also been observed (Sundheim and Krekling, 1982).The mechanism of biocontrol by the fungus has been established as hyper-parasitism as this fungus possess the ability to colonize the mycelium of powdery mildew and produce reproductive structures. *A. quisqualis* parasitic activity was observed in both in sexual and asexual structures of cashew powdery mildew pathogen. It parasitizes and form pycnidia with in powdery mildews hyphae, conidiophores and cleistothecia. This report agrees with earlier studies that revealed mycoparasites suppressing both asexual and sexual sporulation of the attacked powdery mildew mycelia (Sundheim and Krekling,1982). The parasitized colonies of cashew powdery mildew are dull in appearance, flattened off-white to gray in color with reduced spore production. *Ampelomyces quisqualis* parasitism did not stop radial growth of the pathogen but it stopped sporulation of the pathogen. Similarly, Shishkoff and Mcgrath (2002) showed that *Ampelomyces* could not stop the spread of powdery mildew colonies in vitro but did reduce the amount of inoculum produced by each colony.

The percent disease reduction of *A. quisqualis* treatment on seedlings was lower than the positive control; however there was significant reduction of incidence and severity in cashew powdery mildew. This study suggests that including *A. quisqualis* in the integrated pest management will help reduce the disease significantly. *A. quisqualis* has been commercialized as AQ10 and is intended for use as part of an integrated disease management programme and is compatible with a wide range of chemicals (Mcgrath and Shishkoff1999). *Ampelomyces* mycoparasites have been shown to be compatible with a large number of fungicides, acaricides and insecticides used in the control of powdery mildews which makes it possible for their use in integrated pest management programmes (Shishkoff and Mcgrath, 2002).

The sporulation rate of powdery mildew colonies might have been intense in trial II and probably the *A. quisqualis* mycoparasites were slowly following the spread of the pathogen. Therefore mycoparasites reduced the disease incidence to a certain extent. High relative humidity expe-

rienced during the trials *(79-87%) might have enhanced the internal growth and sporulation of *A. quisqualis*. Similar successful biocontrol experiments using *Ampelomyces* were carried out in greenhouses where the relative humidity was kept high (Sundheim *et al.,* 1982 or in the field where free water was frequently available on the treated leaves (Falk *et al.,* 1995).

This study showed that *O. anacardii* is vulnerable to attack by mycoparasite *A. quisqualis.* It demonstrated that through entwining, strangling and penetration processes the pathogen hyphae was parasitized. *A. quisqualis* revealed affinity to the conidia of *O. anacardii*. This study supports the earlier reports that the antagonist is attracted to the host cells by specific chemical stimuli and chemotropic growth (Whipps *et al.,* 1998). The firm attachment observed between conidia of *A. quisqualis* and *O. anacardii* might have probably been as a result of alteration of the pathogen structure (Zelinger *et al.,* 2006). Eziashi *et al.* (2007) observed that the firm attachment between conidia of the *Trichoderma viride* and *Ceratocystis paradoxa* is mediated by a specific cell surface recognition, which in turn, triggers event that leads to host wall penetration. Cell surface molecules play an important role in cell to cell interactions in many biological systems (Inbar and Chet, 1994) and early recognition events, mediated by molecules with sugar-binding affinity, and are known to be important determination in establishing the mycoparasitic relationship between *Trichoderma* species and their target hosts (Benhamou and Chet, 1993).

Ampelomyces quisqualis did not show any observable negative effects on the physiological activities of cashew. Similar results by Romero *et al.* (2003), found that a treatment with *Ampelomyces* significantly increased the chlorophyll content of detached and mildew-infected melon leaves maintained *in vitro.*AQ 10, based on strain AQ 10 of *A. quisqualis* and commercialized in several countries, parasitizes powdery mildew colonies and is active against several powdery mildews on different hosts (Paulitz and Belanger 2001; Copping 2004; Gilardi *et al.,* 2008). Koch's postulates established that *A. quisqualis* is pathogenic to the host powdery mildew. The newly developed pycnidia were cultured and observed to be identical to the mother culture.

The effect of *A. quisqualis* in the control of powdery mildew infections is important in integrated disease management because it suppresses the sporulation rate of its fungal hosts, and the infected plants regain vigour after *Ampelomyces* has killed the pathogens. This mycoparasite has now become one of the most advanced in terms of commercial development of a fungal biocontrol agent for plant pathology as AQ10 Biofungicide. The relationship between cashew plants, powdery mildews and *Ampelomyces* could be further studied from an ecological point of view to understand the role of fungal antagonists in the natural population dynamics of plant parasites**.**

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