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Effektiveness of Bio-spray in the Control of Cashew Powdery Mildew (Oidium anacardii Noack): Shade house and Field Experiments

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TITTEL/TITLE

Effectiveness of Bio-spray in the Control of Cashew Powdery Mildew (*Oidium anacardii* Noack): Shade house and Field Experiments

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SAMMENDRAG/SUMMARY:

Meldugg, forårsaket av soppen *Oidium anacardii*, er en viktig sykdom ved produksjon av cashewnøtter. I dette arbeidet er det sett på effekten av 'bio-spray' (en blanding av utvalgte mikoorganismer og sirup fra casheweple og/eller sukkerrør) mot meldugg og for å finne den optimale konsentrasjonen av middelet mot sykdommen. To forsøk ble gjennomført; i et skygge-hus med småplanter av cashew og på friland i en ordinær planting. Det var fire behandlinger med ulike konsentrasjoner av bio-spray (10%, 15%, 20% and 25%), i tillegg til behandlinger med sirup (uten mikroorganismer tilsatt) i 5% konsentrasjon eller standard fungicider. Et kontroll-ledd var helt ubehandlet, mens et annet var behandlet med vann. I skyggehuset ble plantene inokulert med smitte fra andre planter, mens forsøkene på friland ble lagt i et felt som tidligere hadde hatt mye meldugg. I skyggehuset ble det foretatt to behandlinger med 15 dagers mellomrom bortsett fra fungicid som ble brukt en gang. På friland ble det foretatt fem behandlinger med enten bio-spray, sirup eller vann med 15 dagers mellomrom, mens standard fungicidbehandling ble utført tre ganger med 21 dagers mellomrom. Prosent blad med angrep og angrepsgrad ble registrert i skyggehuset. På friland ble i tillegg fenologisk stadium og avlingseffekter undersøkt. I skyggehuset ble det lite angrep av meldugg, mens det var betydelig angrep på friland. Det såkalte arealet under sykdomskurven (AUDPC) viste at de ulike konsentrasjonene av bio-spray ikke reduserte meldugg sammenlignet med kontroll-leddene. Det var noe bedre effekt av 15% konsentrasjon sammenlignet med 25%. Behandling med fungicider

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var signifikant bedre enn de andre behandlingene og var det eneste forsøksleddet som gav god kontroll av meldugg.

Powdery mildew, caused by *Oidium anacardii*, is an important fungal disease in cashew nut producing countries worldwide. In this study, the effectiveness of 'bio-spray' (a mixture of selected microorganisms and molasses from cashew apple, sugar cane or both) was assessed aiming at determining the optimal concentration of bio-spray and its efficacy in the control of powdery mildew. Two experiments were conducted: one with grafted seedlings in a shade house and one with full size trees in the field. Treatments comprised of four concentrations of bio-spray (10, 15, 20 and 25%), molasses at 5% concentration, and the control treatments (either no application, or treatment with water), and standard fungicides. The occurrence of powdery mildew in the shade house was induced by artificial inoculation on seedlings. The experiment in the field was inside a block of mature cashew trees with a history of high levels of infection by *O. anacardii*, which ensured an adequate supply of natural inoculum. In the shade house there were two treatments with 15 days apart, except from the fungicides that were only applied once. In the field experiment, five applications of bio-spray, molasses and water were carried out at 15-day intervals; however, the standard fungicide treatment consisted of three applications at 21-day intervals. Incidence and severity of powdery mildew were assessed on seedlings in the shade house. Phenological stages, incidence and severity of powdery mildew, and cashew nut scarification and yield were recorded in the field. In the shade house, the success of powdery mildew inoculation was very low, with no difference among treatments. In the field, there were conducive conditions for powdery mildew to reach an epidemic stage. The area under the disease progress curve (AUDPC) showed that the bio-spray concentrations tested did not reduce powdery mildew severity and incidence when compared with the untreated control. However, there was a tendency that powdery mildew progress was relatively lower at the 15% concentration compared to the highest bio-spray concentration (25%). There was a significant difference among treatments on cashew nut yield and scarification of cashew nut shell. Yield was highest on fungicide treated plants and nut shell scarification was lowest on fungicide treated plants. More research is needed on identification of effective microorganisms, multiplication strategies, and storage conditions on the efficacy of the bio-spray.

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KOMMUNE/MUNICIPALITY:	Meconta
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1 Introduction

1.1 Cashew plant

Cashew (*Anacardium occidentale* L.) belongs to the Anacardiaceae family, which includes about 60 genera and 400 species, including mango (*Mangifera indica* L.) and pistachio (*Pistacia vera* L.). Cashew trees are evergreen and reach 8-12 m height (McLaughlin, 2008). The nut of cashew grows outside of the fruit and is attached at the tip of the cashew apple. The cashew kernel grows within the nut. After the nut attains its full size, the fruit become apple-shaped and the nut's shell becomes hard and turns grey. The nut remains firmly attached to the apple and consequently the bulk of the harvest consists of the cashew apples. Nut and apple fall to the ground when fully mature. The nuts are separated from the fruits, sun-dried, and sent to factories for de-shelling and further processing.

Cashew has three valuable parts: the fruit (cashew apple), the kernel (seed) and Cashew Nut Shell Liquid (CNSL). The seed is considered the main commercial part of the cashew tree. The cashew apple can be processed into juice and used to prepare alcoholic drinks. Furthermore, the fruit pulp can be made into jelly, syrup, and molasses. The cashew nut shell contains a reddish-brown, viscous, oily liquid composed of various phenolic lipids, which is poisonous and causes blisters on the skin (McLaughlin, 2008). So, the cashew nut must be carefully processed to extract and remove the liquid of the shell to avoid contaminating the nut. Removal of the kernel from the nuts require a special procedure, and it is labour intensive. Cashew nut shell liquid, which is extracted from the outer shell of the nut, is a by-product of the cashew industry and extensively used in polymer-based industries as a waterproofing agent, preservative, and pesticide (McLaughlin, 2008; Lomonaco, 2009). The cashew seed, fruit and CNSL are commercially valuable.

1.2 Bio-spray

'Bio-spray' contains a living culture of effective microorganisms (EMs). Effective Microorganisms is a mixture of three yeasts, two lactic acid bacteria, three to eight or more actinomycetes and three phototropic bacteria (Tabora and Levi, 2017). The EM technology was started in the 1970's, where beneficial microorganisms were isolated from naturally fertile soils, remixed, and then inoculated into less fertile soils to make them more productive and suppress plant diseases and pests (Higa, 2012). The EMs developed in the 1970's was a mixture of a lactic acid bacterium, photosynthetic bacteria and yeasts, and then fermented with molasses to attain a pH of 3.5 (Higa, 2012). The effective microorganisms are known to work cooperatively to provide benefits for soil and plants (Joshi et al., 2019). Bio-spray is prepared in an anaerobic fermentation by mixing the EMs with molassess (from sugar cane or cashew fruit), some nutrients, and potassium nitrate for one week (Tabora and Levi, 2017).

1.3 Rationale of the study

Cashew is an important commercial crop in several tropical countries worldwide, including Mozambique, and the cashew subsector constitutes an important component for the economy of those countries (Nair, 2010; Zheng & Luo, 2013). Among the main cash crops cultured in Mozambique, cashew plays an essential role in the economy, contributing to more than 3% of GDP (Costa, 2019), creating jobs for more than a million households (Antonio and Griffith, 2017). However, such benefits from the cashew subsector in the economy may decrease due to plant pests and diseases (Majune *et al.*, 2018).

The importance of research and development for the cashew subsector in Mozambique has been shown by continuous improvement of various technologies, which contribute towards solutions for day to day issues arising at farmers' level (Costa, 2019; INCAJU, 2011). Therefore, technological packages that increase cashew nut production and quality must also ensure sustainability of cashew as agribusiness at farmer level and contribute in the best way to the economic development of the country.

Cashew yield and nut quality is affected by biotic factors such as diseases and insects. Among the diseases, powdery mildew of cashew caused by the fungus *Oidium anacardii* Noack (Figure 1A) and anthracnose caused by the fungus *Colletotrichum* sp. pose severe damages to the crop, since they attack young leaves, newly emerging panicles, flowers and young fruits (Freire *et al.*, 2002; Majune *et al.*, 2018). Under suitable conditions, cashew powdery mildew can drastically affect apple and kernel quality, and cause crop losses ranging from 70 to 100% if not controlled (Nene *et al.*, 2017).

For the control of pests, diseases and weeds in Mozambique, an integrated pest management program has been adopted (Costa, 2019), which consists of a set of cropping practices such as weeding, pruning and phytosanitary treatment aiming to increase production, productivity and improve cashew nut quality. However, among the existing control methods for diseases, chemical control has benefited from special emphasis (Majune *et al.*, 2018). This is due to its immediate impact, although the strategy implemented in the country covers less than 25% of the total cashew trees, and chemical control has high administrative and distribution burdens.

In addition, there have been logistic delays from the central procurement to the final spraying service providers, so that the initiation of the pesticide spray program is also delayed and often too late to manage the diseases properly (Figure 1B). Since powdery mildew is a polycyclic disease and most fungicides work preventively, it is difficult to control effectively once it has spread and colonized the tree (Figure 1A).

Figure 1. Powdery mildew on cashew leaves (A) and spraying of fungicides on cashew trees (B). Photo: Mateus J. Comé.

The use of biopesticides are among the alternative control methods that appear promising. For example, Aga Khan Foundation has successfully conducted a series of on-farm trials with a bio-spray program in Cabo Delgado and Nampula Provinces from 2015 to 2016 as 'first-aid' against cashew diseases. The program consisted of an early preventive application of a mixture of effective microorganisms (EMs) and molasses from cashew fruit or sugar cane before flowering, aiming to create unfavourable conditions at the outbreak of powdery mildew. Results were positive since this bio-spray contributed to the increase of production and productivity per cashew tree (Tabora and Levi, 2017).

The bio-spray was also tested in Nampula Province by the Instituto de Amêndoas de Moçambique (IAM-IP), which conducted on-station trials for two seasons (2017/2018 and 2018/2019). In these trials, the effectiveness of the mixture was compared to a negative control or no treatments and a positive control (the standard fungicide triadimenol 250g/L of the product Voltriad at 10 ml dose of the product/cashew tree). An alternate application between bio-spray and the standard fungicide was also included in the trials. In these trials, bio-spray at 5% concentration and 15 days application interval was found to be promising; however, not effective in the control of cashew powdery mildew under higher disease severity in the field. The severity of cashew powdery mildew increased even after the application of bio-spray, although the alternate application between bio-spray and the standard fungicide showed a statistically significant effect ($p<0.05$) in the reduction of severity of powdery mildew compared to the bio-spray used alone. Therefore, the term 'bio-spray' was adopted to indicate the organic function of the mixture and its effect in the reduction of the severity of cashew powdery mildew, as well in the increasing cashew nut production (Tabora and Levi, 2017).

The main challenges identified in previous trials were as follows: (i) the initiation time for flowering vary from season to season and also from one location to another, a reason by which the adequate moment for the first bio-spray application should be identified by scouting shoots; (ii) the concentration of bio-spray tested appeared to be too low to be effective, and therefore, changes would have to be made to test other concentrations greater than 5%; and (iii) the number of days between bio-spray preparation (formulation) and application in the field (solution exposure time in days) is also important, so this would have to be standardized for all treatments.

On this ground, the overall aim of the study was to assess the effectiveness of bio-spray in the control of cashew powdery mildew in a shade house and in the field. The studies aimed at determining the optimal concentration of bio-spray and its efficacy in the control of cashew powdery mildew. In the shade house, the objective was to determine the efficacy of the treatments on seedlings by artificially inoculating the plants. In the field, the study aimed at determining the severity of powdery mildew by use of natural inoculum and on commercial size cashew trees; and the cashew nut scarification (cracking of the outer shell of the nut) level by powdery mildew and yield for each treatment.

2 Materials and Methods

2.1 Description of the study areas

The experiments were conducted at Nassuruma Cashew Research Center located in the District of Meconta, Nampula Province, at latitude 14°58'40.6"S and longitude 39°43'48.2"E (in the shade house) and at latitude 14°59'35.9"S and longitude 39°43'51.7"E (in an on-station cashew orchard). Family farming is one of the most important activities in the district, and the production system is basically rain fed. Cashew plantations are found among other fruit trees as the most predominant crop. Although not technically recommended, the most common cashew production system consists of mixed crooping whereby cashew appears together with cassava, maize and other crops (MAE, 2005).

The experiment was established in the shade house and on-station. The shade house was an inbuilt structure which reduced light by approximately 50% to provide suitable conditions for plant growth (Figure 2A). On-station, the experiment was set inside a block of 15-year-old mature cashew trees (Figure 2B) with a history of high levels of infection by *O. anacardii*, to ensure an adequate supply of natural inoculum.

Figure 2. The study areas: (A) shade house and (B) trial area at the research station. Photo: Mateus J. Comé.

Data regarding daily temperature, relative humidity (RH) and rainfall were recorded during the trial period. The mean temperature in the study area ranged from 18 to 32°C, the RH ranged from 65% to 92%, and low rainfall intensities occurred in August and October 2020, with irregular distribution over time. However, the interaction of factors such as environmental conditions, susceptibility of the crop, associated with the existing inoculum allowed the occurrence of powdery mildew, with severity up to 100% in unsprayed trees.

2.2 Bio-spray preparation

The bio-spray was provided by Jacaranda Monapo; a banana company based in Monapo district and also involved in the development of cashew nut plantations. The Jacaranda Monapo company prepared the bio-spray by culturing microorganisms from a commercial solution of EMs. The EM activation process was made by adding one part of EMs mixed with one part molasses from sugar cane, then fresh milk, yeast and 20 parts water, and finally fermented for one week or until the point when there was no more gas escaping from the container. The fermentation with molasses results in a pH of around 3.8 or lower before dilution and application. The quantity of EMs increased through the fermentation process, and the resulting activated EM (AEM) was ready for application on the cashew trees about two weeks after the first mixing of its components.

The research team was supplied with 20 liters of fresh bio-spray at 100% concentration at each application and a total of 100 liters of fresh bio-spray were supplied for the experiments. The bio-spray was stored at 25°C average temperature.

2.3 Experimental design

The experiments were established in the shade house and in the field, and the same treatments were tested in both experiments (Table 1).

In the shade house, the experiment was established as a Completely Randomized Design (CRD) with eight treatments and four replicates (32 plots in total) (Clewer and Scarisbrick, 2001). Each plot consisted of ten cashew-seedlings about 45 days old and 30 cm average height, from clones susceptible to powdery mildew, namely CCS 23, 11.8PA and 2.4 VM.

In the field (on-station), the experiment was established as a Randomized Complete Block Design (RCBD) with eight treatments and four blocks (32 plots in total) (Clewer and Scarisbrick, 2001). Each plot consisted of five 15-year-old cashew trees.

Table 1. Treatments (T) tested, dilution ratio of the bio-spray, dose of fungicide and number of cashew seedlings and plants used in the shade house and in the field.

No	Treatments description	Dilution or dose	Nº of seedlings in the shade house	Nº of plants in the field
T1	Negative control [a)]	Zero application	40	20
T2	Negative control + water	No dilution or dose	40	20
T3	Molasses 5% concentration	1:19	40	20
T4	Bio-spray 10% concentration	1:9	40	20
T5	Bio-spray 15% concentration	1:5.5	40	20
T6	Bio-spray 20% concentration	1:4	40	20
T7	Bio-spray 25% concentration	1:3	40	20
T8	Standard (chemical products) [b)]	(10ml+5g)/L water	40	20
TOTAL			320	160

[a)] The negative control was not exposed to the experimental treatments or to any other treatments expected to have an effect in the control of powdery mildew.

[b)] The standard consisted of the application of the fungicide triadimenol 250g/L (product: Voltriad 25% EC) + copper oxychloride 850 g/kg (Coprox Super 850 WP).

Water was also used as a negative control to check whether the obtained result was due to the test variable and not the addition of water.

The concentration of bio-spray in the solution used for spraying was obtained by diluting one part of the activated bio-spray in 'x' parts (quantity) of water (1:x), as follows: 1:9, 1:5.5, 1:4 and 1:3 to obtain 10%, 15%, 20% and 25% concentration of bio-spray, respectively. The same principle was used to obtain the treatment consisting of molasses at 5% concentration (Table 1). The standard fungicide treatment consisted of Voltriad 25% EC at 10 ml dose/liter of water mixed with Coprox Super 850 WP at 5g dose/liter of water in the same tank.

In the shade house, only two applications of bio-spray at 15 days interval, and one application of the standard fungicide treatment were made.

For the field experiment, the start of application was determined by assessment of phenological stage of the trees combined with powdery mildew incidence. Thus, the spraying began when approximately 10% of the panicles had emerged and with a mean severity of powdery mildew less than 5%. Five applications were carried out with the bio-spray, molasses and water at 15-day intervals. The standard fungicides were applied three times at 21-day intervals, as per the guide on use and application of the pesticides or established norm in the management program of pests and diseases in use in the country.

2.4 Inoculation of seedlings in the shade house

Seedlings were artificially inoculated with powdery mildew by brushing conidia from naturally diseased leaves onto flushing young leaves of cashew seedlings to ensure deposition of inoculum (Cardoso *et al.*, 2017).

Diseased leaves from infected cashew trees were collected in the field, put in kraft papers and brought to the shade house the same day for inoculation of susceptible cashew seedlings (clones CCS 23, 11.8PA and 2.4 VM). These were used as source of fresh inoculum for the experiment, to ensure that conidia were of a similar stage of maturity when inoculating the target-seedlings in the trial.

One day before inoculation of target-seedlings with *O. anacardii*, experimental treatments (Table 1) were applied on seedling leaves. The seedlings were artificially inoculated as described above, with powdery mildew from seedlings used as source of young inoculum, and they were covered with thin plastic for 48 hours after inoculation to maintain high humidity to increase infectivity (Alfenas et al., 2016). To further stimulate disease development, humidity was increased by watering the ground twice a day (in the morning and afternoon) throughout the experimental period. Daily information on temperature and humidity was recorded at the research station where the experiment took place.

2.5 Data collection

Incidence and severity of powdery mildew on seedlings in the shade house were assessed on five first true leaves counted from the tip of the seedling. Each seedling was coded on a tag tied to the seedling stem collar (Figure 3A). The assessment of incidence and severity of powdery mildew was done twice a week and only for two weeks, since there were no visible symptoms of powdery mildew developing. The disease incidence in the shade house was obtained as percentage of seedlings infected in the experiment.

Assessments on established cashew-trees in the field were carried out on 10 shoots per cashew-tree, five on the north side and five on the south side. Plants in the experiment were coded (Figure 3B), and shoots or panicles labelled using a sisal rope of 20 cm length for identification (Figure 3C) during plant inspection and powdery mildew scoring (3F).

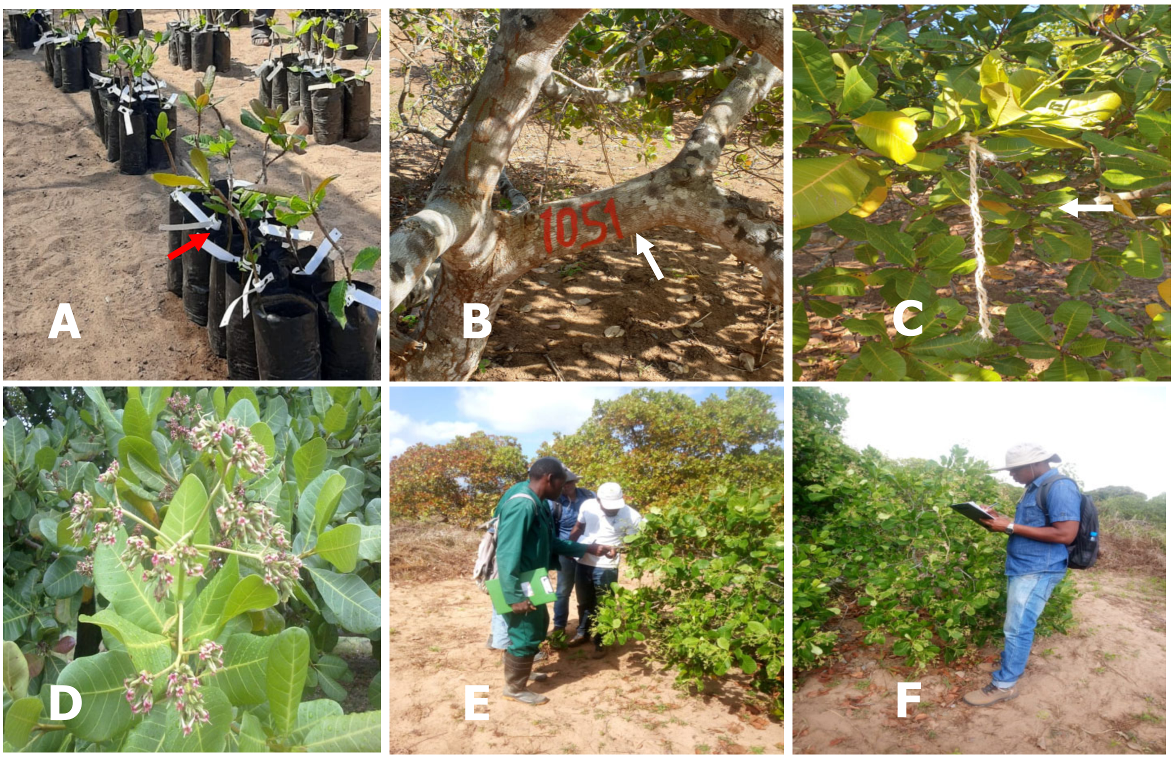

Figure 3. Plants and seedlings selected for the experiments. (A) Seedlings labelling in the shade house. (B) Plant coding in the field. (C) Shoots or panicles labelling in the field. (D) Cashew panicle. (E) Inspecting plants for disease. (F) Powdery mildew scoring. Photo: Mateus J. Comé.

The assessment of phenological stages, incidence and severity of powdery mildew was done once a week in the field, for twelve weeks, using visual diagrams or assessed by visual estimation of the leaf area colonized by powdery mildew (Adiga *et al.*, 2019).

Phenological stages (Figure 4) were assessed by means of a diagrammatic scale with levels ranging from 1 to 8; where 1 = stage of intumescence development of leaves, 2 = leaves start budding, 3 = red leaves flush, 4 = beginning of panicle formation, 5 = opening of flowers, 6 = beginning of fructification, 7 = full fructification and 8 = fruit maturing stage.

The powdery mildew severity (proportion of the total area of plant tissue affected by disease) on leaves of seedlings in the shade house and cashew blossoms of panicles in the field was assessed using a diagrammatic scale adapted from Nathaniels (1996), with levels ranging from 0 to 6; where 0 = no infection; 1 = (0 - 10%), surface of leaves showing epiphytic mycelium of powdery mildew, demonstrating the active sporulation; 2 = (10 - 25%); 3 = (25 - 50%); 4 = (50 - 75%); 5 = (75 - 99%); and 6 > 99%. The disease severity was calculated as follows:

$$\text{DSI (\%)} = \frac{\sum_0^n (\text{No. of plants with powdery mildew symptoms in each class} \times \text{mid-point of disease scale})}{\sum_0^n (\text{Total number of plants assessed})}$$

; where DSI = Disease Severity Index, and n = number of shoots or panicles observed in a dataset.

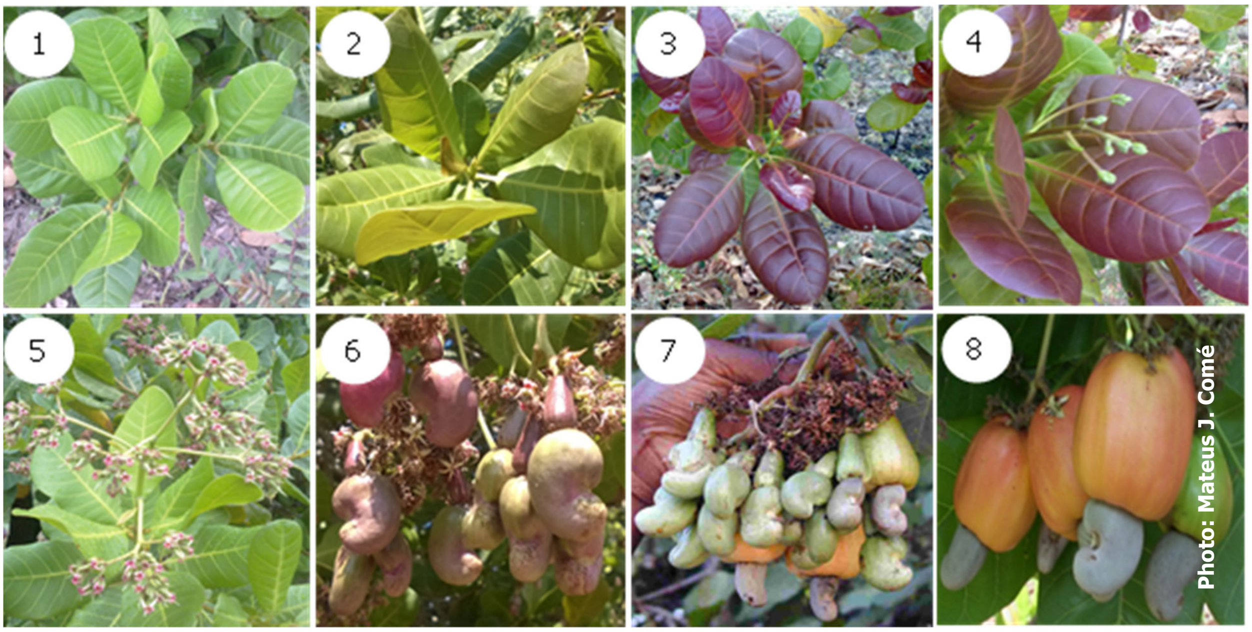

Figure 4. **Diagrammatic scale for estimation of cashew phenological stages; 1 = stage of intumescence development of leaves, 2 = leaves start budding, 3 = red leaves flush, 4 = beginning of panicle formation, 5 = opening of flowers, 6 = beginning of fructification, 7 = full fructification and 8 = fruit maturing stage. Photo: Mateus J. Comé.**

2.6 Data processing ang analysis

The Microsoft Office Excel package and MINITAB version 19 were used for data processing and drawing of graphs on phenological stages, disease progress and for statistical analysis. The area under the disease progress curve (AUDPC) used to summarize the progress of disease severity over time was estimated considering the number of times the disease severity was evaluated, the disease severity at each evaluation time and the time duration of the epidemic in the experiment (Hikishima *et al.*, 2010). The AUDPC was calculated using the trapezoidal method as described by Madden *et al.* (2007). MINITAB version 19 was used for Analysis of Variance (ANOVA) at 5% significance, after verifying the assumptions for ANOVA. Treatment means were compared by Tukey's test ($p<0.05$) for each of the following parameters: phenological stage, incidence and severity of cashew powdery mildew, AUDPC of disease severity and incidence, and cashew nut scarification by powdery mildew.

3 Results and Discussion

3.1 Incidence and severity of powdery mildew on seedlings

The success of powdery mildew inoculation and further disease development in the shade house was very low. Only 82 of 320 (26%) seedlings showed any symptoms of powdery mildew three days after inoculation, with less than 5% severity for about a week (very low severity), and symptoms then disappeared. The low success of the inoculation process was likely because the shade house conditions did not allow a satisfactory control of temperature, humidity and air movement in the course of the experimental period. Although the relative humidity in the study area was sufficiently high for the seedlings to get infected, the temperature was too high, with an average over 30˚C, which did not allow conidia to germinate and infect the seedlings.

In addition to the unfavourable environmental conditions for the pathogen in the shade house, the rapid development of age related (ontogenic) resistance on cashew leaves (within 7 to 14 days) may also have limited the infection success, colony expansion, sporulation and disease development of *O. anacardii*.

Studies reported that environmental conditions in the trial site, mainly the temperature and relative humidity, must be controlled for successful infection and reproduction of powdery mildew after inoculation on susceptible plants or plant parts (Cardoso *et al.*, 2017; Dominic and Makobe, 2016; Majune *et al.*, 2018; Swart, 2004). Swart (2004), for example, conducted a pathogenicity test with cashew plants maintained in pots on open benches in a glasshouse. Environmental conditions in the trial site were controlled; the mean relative humidity was 65% and the temperature ranged from 22^{o}C to 25^{o}C.

In this trial, *O. anacardii* inoculated on seedlings did not persist for more than a week, likely because of unfavourable environmental conditions and leaf age related resistance. The low success of the artificial inoculation in the shade house could also be due to the quantity and purity of the inoculum.

3.2 Phenological stage in the field

Table 2 shows the phenological stages in the field, represented by the most frequently occurring value in the set of scores per week (data recorded weekly). There was a slight difference among treatments on phenological stages of the plants, mainly during the first four assessments. For example, at the first registration point, most plants were at the red leaves flush stage for some treatments (stage #3), while others were at the beginning of panicle formation (stage #4) for other treatments (Table 2). This difference in the rate of change of the phenological stage among the treatments may have had some implication on the initiation of the disease development.

Phenological stages from 3 to 5 describe plants from the red leaves flush to the opening of flowers. These stages were relatively delayed for about four weeks in the 2020 season, since the red leaves flow occurred from late June in Nampula Province. The flowering stage started in mid-July for most plants on-station.

According to Martins *et al.* (2016), *O. anacardii* attacks young tissues, and the incidence and severity vary from stage to stage, where the red leaves flush, beginning of panicle formation, opening of flowers and beginning of fructification are the most affected. In this study, phenological stages showed that plants in the field passed through all stages described as potentially susceptible for powdery mildew (Majune *et al.*, 2018).

Table 2 also shows that the data scoring in the field started at the stage # 3 (red leaves flush) for most treatments, and the panicle formation started a week after the establishment of the trial. Flowers opened within two weeks after the panicle emergence in all treatments. Then, most of the plants of the standard treatment consisting of the Voltriad and Coprox turned into the fruit set stage five weeks after the opening of flowers, but plants treated with the bio-spray a few of them turned to the fruit set stage.

Table 2. The most frequently occurring (mode) phenological stages in the set of scores (data recorded weekly over 12 weeks)

Number of registration weeks	TREATMENT *							
	NEGATIVC	NEGWATER	MOLASS5	BIOSPR10	BIOSPR15	BIOSPR20	BIOSPR25	STANDARD
1	3 (50%)	3 (67%)	4 (64%)	3 (47%)	3 (38%)	3 (48%)	4 (53%)	4 (54%)
2	4 (87%)	4 (73%)	4 (93%)	4 (51%)	4 (52%)	4 (68%)	4 (70%)	4 (81%)
3	4 (60%)	4 (73%)	4 (81%)	4 (47%)	4 (43%)	4 (49%)	4 (59%)	4 (50%)
4	5 (53%)	4 (55%)	4 (54%)	5 (41%)	5 (57%)	5 (50%)	5 (62%)	5 (70%)
5	5 (85%)	5 (70%)	5 (94%)	5 (59%)	5 (69%)	5 (79%)	5 (77%)	5 (87%)
6	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	6
10	5	5	5	5	5	5	5	6
11	5	5	5	5	5	5	5	6
12	5	5	5	5	5	5	5	6

** Details about treatments are shown in Table 1 and about the phenological stages in Figure 4.*

The occurrence time and duration of each phenological stage varied slightly for each treatment. After reaching the opening of flowers (Stage #5), this stage lasted to the end of the trial period as the most frequent phenological stage for all treatments, except the standard treatment (Table 2). This pattern was likely because the severity of powdery mildew was low only in the standard fungicide treatment; the other treatments did not reach stage 6.

According to Martins *et al.* (2018), the progress of powdery mildew in cashew is associated with the density of the inoculum in the trial site, environmental factors and to the susceptibility of plant organs to the pathogen. This susceptibility may vary from organ to organ (Martins *et al.*, 2016); however, greater variation is recorded from one phenological stage to another. This finding shows that phenological studies are important for the understanding of the development of growth stages in disease management.

3.3 Effect of bio-spray on the progress of powdery mildew in the field

Powdery mildew severity increased over time in all treatments, except for the standard fungicide treatment (Figure 5). Treatments with bio-spray and molasses, as well as the negative control and the treatment with water could not suppress the disease and control the development of symptoms. From late October 2020, the severity of powdery mildew on plants treated with molasses and bio-spray at 25% concentration and untreated cashew trees stabilized around 85% in average (Figure 5). No further disease development occurred, likely because temperature was high (over 30°C in average) and the relative humidity was less than 80% in the study area. According to Majune *et al.* (2018), conducive environment for cashew powdery mildew is temperatures from 25°C to 28°C (optimum 26°C), and relative humidity from 80% to 100% (optimum 95%).

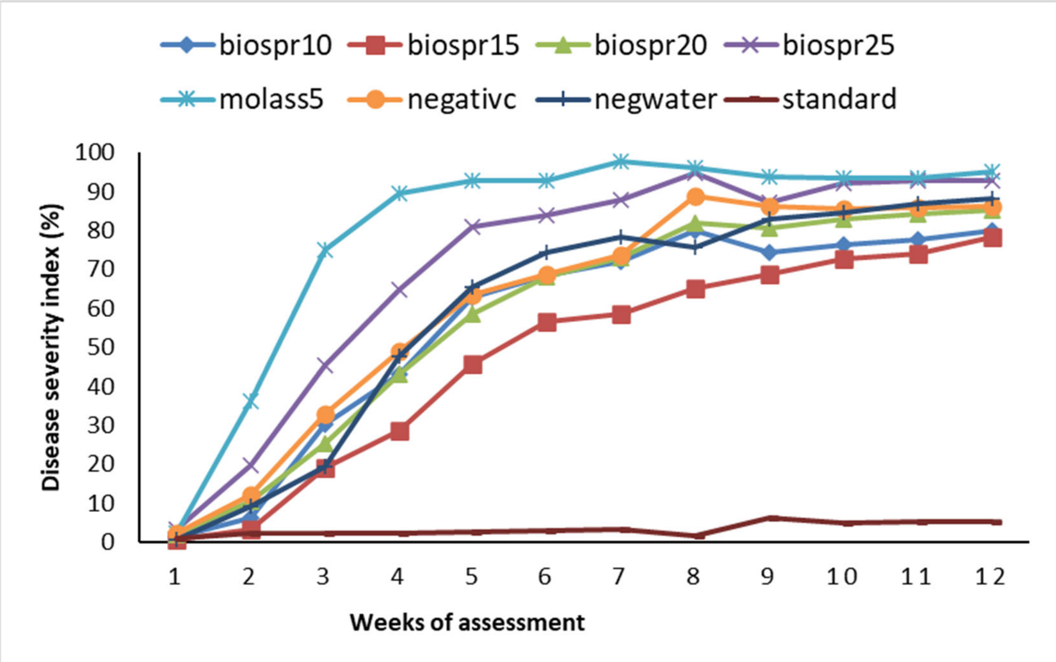

Figure 5. Effect of the different treatments on the progress of powdery mildew severity. The first scoring (week 1) was at 29 July 2020 and the following were made weekly.

There was adequate supply of natural inoculum of *O. anacardii* for the plants. The incidence of powdery mildew in the trial increased progressively from less than 20% in the first scoring at the beginning of the experiment for the majority of treatments, except for the bio-spray at 25% concentration, which showed over 30% incidence at the first assessment.

The area under the disease progress curve (AUDPC), which summarized the progress of powdery mildew in the trial, is presented as disease severity and disease incidence (Table 3). Compared to the non-sprayed control plants, none of the bio-spray concentrations reduced powdery mildew severity and incidence. When compared within the bio-spray concentrations, the treatment at 15% concentration showed the lowest disease incidence (Table 3).

For disease severity, the standard fungicide treatment showed the lowest AUDPC, with significant difference from all other treatments. Comparing AUDPC based on the disease incidence data, there was no significant difference between the bio-spray at 15% concentration and the standard (Table 3). It should be noted that disease incidence did not show the level of damage on the plants. Among treatments with bio-spray, most concentrations did not show significant differences; however, the bio-spray at 15% concentration had a lower disease level compared to 25% concentration. Treatment with molasses at 5% concentration gave the highest disease severity in the trial (Figure 5 and Table 3). The high powdery mildew incidence in the molasses treatment could be associated with the rapid change in phenological stage (Table 2) of the plants (stage 3 and 4, which are the most susceptible stages to powdery mildew) and build-up of the inoculum early in the season. According to Martins *et al.* (2016), *O. anacardii* usually attack young tissues, and the incidence and severity is high when the infection occurs in the stage 3 (the red leaves flush), stage 4 (beginning of panicle formation), stage 5 (opening of flowers) and stage 6 (beginning of fructification) (Figures 4).

Table 3. Effect of the treatments in the whole disease assessment period as summarized by the area under the disease progress curve (AUDPC) for powdery mildew disease incidence and severity.

Treatments	AUDPC (incidence)*	AUDPC (severity)*
Molasses @ 5% concentration	6 885 A	6368 A
Bio-spray @ 25% concentration	6 393 AB	5582 AB
Negative control	5 754 ABC	4836 ABC
Negative control + water	5 532 ABC	4683 BC
Bio-spray @ 20% concentration	5 460 ABC	4564 BC
Bio-spray @ 10% concentration	5 140 BC	4425 BC
Bio-spray @ 15% concentration	4 734 CD	3726 C
Standard fungicides	3 504 D	257 D
p-value	< 0.001	< 0.001

* *Means that do not share a letter are significantly different by Tukey's test at 5% probability.*

Panicles of plants treated with the standard fungicides remained healthy (Figure 6A-B) and turned into the fruit set stage with fruits free of powdery mildew (Figure 6C-D). Panicles of cashew trees treated with bio-spray showed mean severity of around 80%; however, they stayed alive to the end of the trial period (Figure 6E-G). Molasses applied alone contributed to an increase in disease severity in the field and to death of panicles (Figure 6H).

Panicles of the trees treated with molasses showed high disease severity (severity over 90% in some plots) and died about four weeks after the opening of flowers, thus showing a lower frequency of plants with flowers that turned into the fruit set stage (Figure 6H) or stages 6 and 7 (Table 2, Fig. 4), with significant flower loss as also reported in other studies (Freire *et al.*, 2002; Majune *et al.*, 2018; Nene *et al.*, 2017). It is not clear why the trees treated with molasses alone contributed to an increase in powdery mildew severity and death of panicles in the field.

Figure 6. (A-D) Healthy panicles and fruits from plants treated with the standard treatment. (A) Beginning of panicle formation stage. (B) Panicle at the opening of flowers stage. (C) Panicle at the beginning of fructification stage. (D) Healthy fruits under formation (fructification stage). (E-H) Typical panicles and fruits from plants treated with bio-spray, molasses, untreated plants and water-treated plants. (F) Panicle treated with bio-spray, alive, although showing high severity of powdery mildew, regardless the concentration. (G) Typical panicle at the fructification stage showing high severity of powdery mildew. (H) Typical panicles treated with molasses, which eventually died. Photo: Mateus J. Comé.

Although the bio-spray did not provide good efficacy against powdery mildew in the present study, there are reports that the EMs can suppress soil borne pathogens in vegetable crops. The positive effect of EMs in vegetables is claimed to be by increasing the microbial diversity of soil ecosystems, and by improving and maintaining the soil chemical and physical properties (Iriti *et al.*, 2019; Joshi *et al.*, 2019; Samy, 2019). Most studies report that EMs had a positive effect on the growth of vegetables and control of pests and diseases (Joshi *et al.*, 2019); however, others indicate that EMs might not be effective (Olle and Williams, 2013; Iriti *et al.*, 2019). In this study, the severity of powdery mildew in the treatments with bio-spray increased progressively up to 70% in average (Figure 5).

3.4 Cashew nut scarification and yield

In general, the cashew nut yield per tree was very low for all treatments. None of the treatments reached the minimum historic recorded yield in the station. Historical information shows that yields of various cashew clones planted in the field where the experiment took place at the Nassuruma Cashew Research Center were from 5 to 25 kg/tree. In the present study, the yield was lower than 1.2 kg/tree. The number of nuts were not sufficient to take representative samples for the scarification assessments, so yield and scarification data were thus not included in this report. Based on the available data, there was a tendency that on the fungicide treated trees, the nut yield was higher and cashew nut shell scarification was lower, which means less powdery mildew damage on the nut. According to Serrano *et al.* (2013), the scarification is used to estimate the effect of powdery mildew on the quality of cashew nuts, and when the cashew nut scarification increases the quality decreases at the same rate.

Use of EMs has increased yields with up to 50% in vegetables (Higa, 2012). The EMs act as a very effective plant growth promoter in some crops such as common oak, cucumber, pumpkin and squash transplants, as well as a stimulant of flowering and foliage growth when applied before flowering (Bzdyk *et al.*, 2018; Olle and Williams, 2013; Olle and Williams, 2015). In the earlier demonstration plots trials, it was found that bio-spray increased the cashew nut yield and maintained the quality of seeds (Tabora and Levi, 2017).

As reported in other studies, the components of the bio-spray are more effective when applied in the soil with organic matter (Higa, 2012), so it may be better to target the bio-spray on soil borne pathogens of cashew and vegetables. The success of the EMs may increase if applied with nutrient rich organic matter to make a fermented compost, and it promotes the release of nutrients and nutrient rich organic acids, which may be utilized by plants (Higa, 2012). The benefit to the plant health may be because EMs develop a positive influence both to the plant and the micro and macro climate surrounding the rhizosphere of the plant, which in turn help the plants to tolerant diseases and pests.

4 Conclusion

The bio-spray concentrations tested in this trial did not reduce powdery mildew severity and incidence when compared with the untreated control. However, when comparing within the bio-spray concentrations, there was a tendency that powdery mildew progress were relatively lower at the 15% concentration than the highest bio-spray concentration (25%). This 15% concentration may thus be the optimal and be used in further experiments to clarify the efficacy of the bio-spray for example in combination with other alternatives or by reducing the application intervals from 15 days.

5 Recommendation

A possible beneficial effect of effective microorganisms (EMs) in the control of powdery mildew and perhaps other diseases of cashew need to be studied further. We do not know the mode of action of the EMs on the target pathogens, and we also need more knowledge on the effect of type of molasses on multiplication and activation of the EMs, the impact of pH and storage conditions. A possible phytotoxic effect of the molasses also need to be studied further. Finally, the optimal number of days between bio-spray preparation (formulation) and application in the field need to be determined.

Powdery mildew is a devastating disease of cashew, so research on alternative control measures are crucial for developing a sustainable production. Therefore, more research need to be carried out that includes other environmentally friendly products, such as sulphur, AQ10 (*Ampelomyces quisqualis*) Serenade (*Bacillus* sp.) and other products that have shown good efficacy against powdery mildews in other crops. Bio-spray could be alternated with such alternative disease management practices.

Capacity building for researchers, technicians and growers involved in the cashew sector, to increase the understanding of pathogen biology, epidemiology, windows of cashew susceptibility, timing of control measures and sustainable disease management practices is also important.

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