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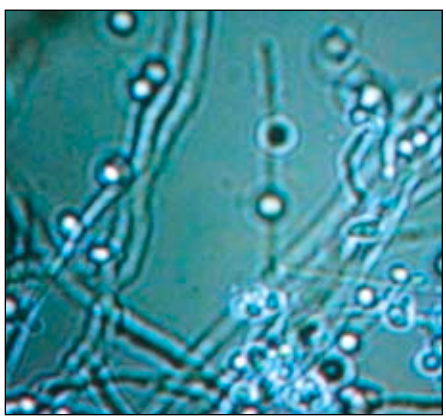
ASSESSMENT OF DIFFERENT MEDIA FOR THE PRODUCTION OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA*

TO CONTROL THE RED PALM WEEVIL RHYNCHOPHORUS FERRUGINEUS (OLIV.)

Keywords: *Beauveria bassiana*, culture, media, conidia, *Rhynochophorus ferrungineus* , red palm weevil, bioassay.

ABSTRACT

The growth of the entomopathogenic fungus Beauveria. bassiana (B-SA3) in different media and subsequent quantity and viability of produced conidia was determined as well as virulence against the red palm weevil R. ***ferrungineus***. Five ingredients in agar were tested; wheat, corn, barley, oat and soy bean. The standard SDYA medium was used for comparism.

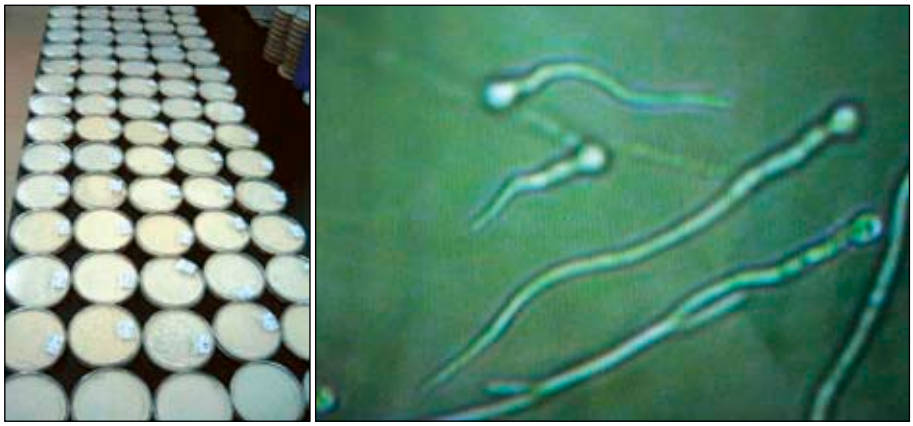


The yield of cultured conidial was highest on media prepared by wheat followed oat and least on barley. Viability of harvested conidia from all of the tested media, as determined by percentage germination ranged between 94.03-97.6% and relatively comparable with those obtained using SDYA. B.bassiana (B-SA3) conidia produced on the tested media although highly infective to R. ***ferrungineus*** adults was significantly less than that harvested from SDYA as exhibited by the calculated LC50 and LT50 . Of the considered ingredients, conidia cultured on wheat medium surpassed those produced on the other media (LC50 was 2.11x 107 conidia / ml) followed by soy bean (1.06x108 conidia / ml). Least infectivity to the red palm weevil was by conidia produced on oat or corn medium, as LC50 was 2.81x 108 and 3.37x 108 conidia / ml, respectively. Mycosis was apparent on cadavers by the 7th day following death of all treated weevils.

INTRODUCTION

The date palm trees are infested by many insect pests as well as bacterial and fungal diseases leading a great loss in their harvest and sometimes to damage beyond repair to the trees. In the past two decades the red palm weevil, ***Rhynchophorus ferrugineus*** (Oliv.) (Coleoptera: Curculionidae) has become a major key insect pest to date palm trees. This insect pest has been widely accepted as being the most devastating insect pest of date, coconut and oil palm trees throughout Asia (Kalshoven, 1950; Wattanaapongsiri, 1966 and Abraham et al., 2002). In the mid 1980's R. ***ferrugineus*** was introduced to date palm trees in the Arabian Gulf Region. It quickly spread to Saudi Arabia, Iran and Egypt (Abraham et al., 1998; Murphy and Briscoe, 1999 and Soroker et al., 2004). In 1997, the Arab Organization for Agriculture Development, AOAD, based in Sudan set a project with the aim of controlling this destructive insect pest.

Due to the insect feeding habits inside the palm tree trunks, its control has been quite difficult; furthermore, early infestation can not be discovered until damage has already been inflicted. Efforts for the control of the red palm weevil were focused on the use of traditional chemical insecticides or by eliminating infested trees. The use of food baited pheromone traps in both surveillance and mass trapping form a vital component of an IPM strategy against the red palm weevil (Abraham et al., 2002). Control of this pest is now more concerned with the use of biological control agents, such as the use of entomopathogenic bacteria, viruses, fungi or nematodes. Beauveria bassiana is well known as an entomopathogenic fungus with worldwide distribution with broad spectrum insecticidal activity (Li, 1989 ; Donaldson and Williams, 1981; Martin et al., 2000; Saleh and Alheji, 2004). This fungus is the anamorphic stage of Cordyceps bassiana, a teleomorph in the ascomycetous family, Clavicipitaceae (Sung et al., 2007). B. beauveria has



proven to be effective for the control of many Coleopteran species, (Miranpuri et al., 1992a and 1992b, Miranpuri and Khachatourians, 1994; Magra et al., 2004).

Use of entomopathogenic fungi in a bio-control program will require production of large amounts of inoculums. The components of the Sabouraud's dextrose yeast agar (i.e. SDYA) medium as a culture for the entomopathogenic fungus B. bassiana although very successful is relatively costly. Therefore, for the mass production of B. bassiana it was significant to experiment with other less expensive ingredients in the cultivation medium. Subsequently, the produced B. bassiana conidia on these media were evaluated in regard amounts obtained, viability and virulence on the red palm weevil.

MATERIAL AND METHODS

Insect culture of the red palm weevil Rhynchophorus *ferrugineus* (Oliv.) :-

The culture of the red palm weevil Rhynchophorus ferrugineus (Oliv.) was conducted under laboratory conditions at 27±20C and 70±5 % RH. Adult weevils were collected from infested date palm trees at a plantation located at El Kassasine, Ismailia Governorate, Egypt, by means of insecticide free food baited aggregation pheromone / kaironome traps (Hanounik et al; 2000). The traps were partially buried around the trunk of the date palm trees at distances of 100 m. The traps were routinely inspected and the live trapped weevils were collected and transferred to the laboratory and reared according to Aldossary et al. (2009).

The Entomopathogenic Fungus *Beauveria bassiana* (B-SA3):-

The entomopathogenic fungus Saudi Arabian isolate (B-SA3) of B. bassiana was used in the present investigation. This fungus was isolated from dead red palm weevils R. ferrugineus collected from date palm plantations at Al-Qatif province, at the Kingdom of Saudi Arabia by Hegazy et al. (2007) and which was identified and confirmed by CABI Bioscience UK.

Mass cultivation of *Beauveria bassiana* (B-SA3):-

Five seeds or grains, namely oat, barley, wheat, corn and soy bean were chosen to prepare a media for the cultivation of B. bassiana. The seeds or grains were thoroughly washed in running water and then allowed to dry at room temperature; subsequently they were grounded to a fine powder by means of an electric mill. For the preparation of the media two weights, 20 or 40 gm per liter were considered for each tested ingredient, the grounded powder was mixed with 15 gm agar per liter of distilled water and autoclaved at 1210 C for 20 min. The standard Sabouraud's dextrose yeast agar (i.e. SDYA) culture medium was used as a standard to compare the performance of the considered media.

The fungus B. bassiana (B-SA3) was grown on SDYA as well as the five other media for 96 hr at 25±20C, subsequently 5 mm in diameter discs were taken from the active growth region of the fungal colony and transferred in the centre of a 10 cm Petri dish containing 20 ml in each of the prepared culture media to be tested. Each considered culture medium was presented by 3 plates and each replicated 4 times. After incubation at 25±20C for 15 days, the conidia were harvested in sterile vials by scraping the surface of agar plates and the following factors were determined:-

(a) Dry weight (gram) of produced conidia/plate.

(b) Viability of conidia, assessed by germination test according to Goettel and Inglis (1997).

(c) Sporulation of the fungus was determined by conidial counting as number of conidia/cm2 of fungal growth. After incubation at 25±2°C for 15 days, conidia were harvested by flooding the sporulated culture with sterile distilled water containing 0.05 % Tween-80 and dislodging conidia with a glass rod. Final conidial suspension volume was 25 ml and that was vortexed for 2 min to break spore chains into individual spores to assure uniform mixing. Counting of conidia was conducted by the use of a hemocytometer slide under an optical microscope.

(d) A bioassay of the B. bassiana conidia cultured on each of the considered media ingredient (i.e. corn, wheat, oat, soy bean or barley) as well that cultured on the standard SDYA medium was evaluated on adult R. ferrungineus weevils. A series of concentrations were prepared, 5x105, 5x106, 5x107, 5x108 and 5x109 conidia/ml of B. bassiana according to the method described by Marannino et al. (2006), the suspensions were used for inoculation within 1 hour. The dipping technique was used, where 20 weevils were dipped for 20 seconds in one of the prepared concentrations. Treated specimens were then maintained separately and provided with small pieces of date palm wood as a source of food; each treatment was replicated 3 times. A control present by an equal number of weevils was included. Accumulated mortality percentage of treated weevils was recorded and corrected according to Abbott's formula (Abbott, 1925) and LC50 and LT50 values determined (Finney, 1952). Following the death of treated weevils their cadavers were incubated individually at 250 C in sealed moist Petri dishes. After 7 days the specimens were examined to determine if the death was a result of infection by the fungus as confirmed by mycosis and/or 'mummification'.

Ingredient	N°. conidia/ cm^2 cultured on media in two weights (gm / liter)	
	20	40
Oat	5.5x 10^8	7.9x 10^8
Wheat	6.4x 10^8	7.6x 10^8
Corn	3.1x 10^8	4.3x 10^8
Soy bean	3.3x 10^8	5.5x 10^8
Barley	2.9 x 10^8	3.1x 10^8
SDYA	8.7x 10^8	

Table (1): Number of B. bassiana (B-SA3) conidia/ cm2 grown on media prepared with different ingredients.

RESULTS AND DISCUSSION

1-Weight of produced B. *bassiana conidia*.

As seen in Fig. 1 the medium prepared with oat or wheat produced the highest weight of B. bassiana conidia. Wheat medium was slightly superior when added at 20 gm than oat medium, as an average weight of 0.138 and 0.127 gm of conidia were obtained, respectively, however these values were significantly lower to conidia cultured on SDYA. Meanwhile, when the medium for the culture of conidia was prepared with 40 gm of either of these two grains, the weight of cultured B. bassiana conidia was 0.176 gm in the medium made with oat which was only slightly but insignificantly higher than that of the medium prepared with wheat, i.e. 0.171 gm. It is noteworthy, that the weight of conidia produced

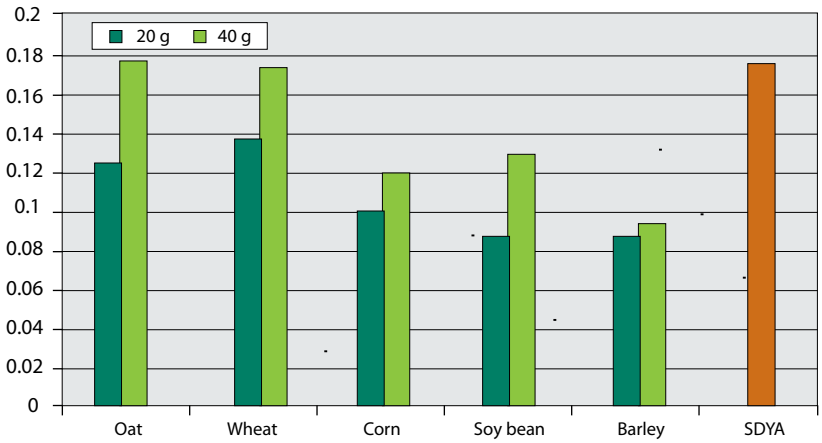


Fig. (1): Average weight of B . ***bassiana*** (B-SA3) conidia (gm) cultured on media prepared with different ingredients

on either of these two medium was also insignificantly different than those produced on the standard SDYA medium (i.e. 0.174 gm).

The medium made by 20 gm of corn, barley or soy beans produced a markedly lower weight of conidia than the standard SDYA medium, i.e. 0.097, 0.089 and 0.086 gm, respectively, as compared to 0.174 in SDYA; the difference between them was highly significant. Meanwhile, when 40 gm of these considered ingredients were added, the weight of cultured conidia was higher in the medium containing soy bean followed by that made with corn and least in barley, i.e. 0.132, 0.122 and 0.092 gm, respectively.

2- Number of *B.bassiana* conidia / cm2

The number of B. bassiana (B-SA3) conidia/ cm2 obtained when cultured on the different tested media varied (Table 1) . The standard SDYA medium was the most efficient in producing the largest number of B. bassiana conidia i.e. 8.7 x 108 conidia/ cm2 .

Of the tested media that prepared with 40 gm of oat or wheat produced the highest number of B. bassiana (B-SA3) conidia which was 7.9x108 and 7.6x108 conidia/ cm2, respectively. However, when either of these media's were prepared at a lower weight of 20 gm, that made with wheat was slightly superior to that of oat as the number of conidia was 6.4x108 and 5.5x108 conidia/ cm2, respectively .

Media prepared with 20 gm of either soy bean or corn were similar in regard the number of cultured conidia being 3.3x108 and 3.1x108 conidia/ cm2, respectively. However, when prepared with double their quantities i.e. 40 gm, of either soy bean or corn the former medium produced 5.5x108 conidia/ cm2 which was much more than those produced on corn (4.3x108 conidia/ cm2).

The number of produced B. bassiana (B-SA3) conidia cultured on a medium prepared with either 20 or 40 gm barley was the lowest as only 2.9x108 and 3.1x108 conidia/ cm2 , respectively, were counted.

3- Germination test of Beauveria bassiana (B-SA3) conidia cultured on media's prepared with different ingredients:-

In the standard SDYA medium percentage of conidia germination was 98.2 %, as seen in Fig. 2, the germination of conidia cultured on any of the tested media ranged between 94.03-97.6%. Highest percentage of germination was detected in B. bassiana conidia cultured on a medium prepared with 40 gm of oat, wheat or corn, i.e. 97.6, 97.6 and 97.5 %, respectively. These percentages were comparable and insignificantly different than that of B. bassiana conidia harvested from SDYA medium. When the media were prepared with these mentioned ingredients (i.e. oat, wheat or corn) but at a half their weight (20 gm) percentage of conidia was slightly reduced to reach

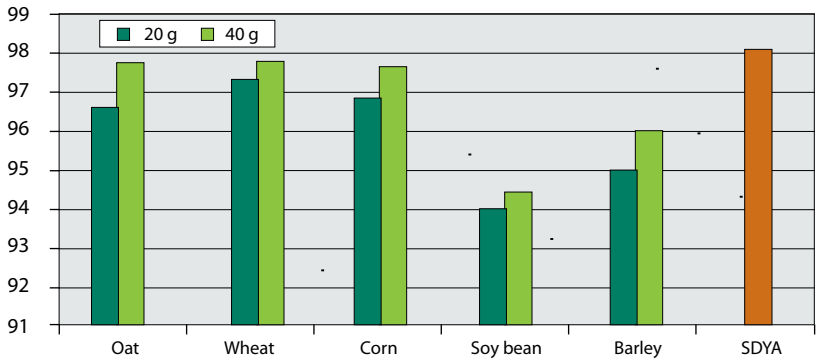


Fig. (2) Germination percentage of B. ***bassiana*** (B-SA3) conidia cultured on media prepared with different components

96.5, 97.3 and 96.7 %, respectively. These results show that wheat medium was the least affected when prepared in lesser amounts. Lowest germination was detected in conidia cultured on soy bean medium, prepared in either 20 or 40g, being 94.03 and 94.4 %, respectively, which was significantly less than germination of conidia harvested from SDYA medium.

4- Bioassay of *Beauveria bassiana* (B-SA3) conidia cultured on different media:-

The virulence and infectivity of B. bassiana (B-SA3) conidia depicted by accumulative percentage mortality of adult red palm weevils treated with different concentrations of B. bassiana (B-SA3) conidia harvested from different media are exhibited in Fig. 3 & 4 and were shown to be concentration dependant. Accumulated mortality was highest in red palm weevil treated by B. bassiana (B-SA3) conidia harvested from the media prepared with wheat followed by barley then soy bean in any of the tested concentrations.

The previous observation was confirmed by the determined LC50 values which were 2.11x107, 6.9x107 and 1.06x108 conidia/ml in the media prepared by 40 gm of wheat, barley or soy bean, respectively, (Table 2). Meanwhile, when these respective mentioned ingredients when included in lesser amounts of 20 gm, the LC50 values were higher, being 5x107, 1.96x108and 2.37x108 conidia/ml, respectively. However, conidia collected from the standard SDYA were much more infective to red palm weevil adults as a lower LC50 value of 3.75 x 106 conidia/ml was calculated, (Table 2).

Least infectivity was detected in red palm weevils treated with B. bassiana (B-SA3) conidia harvested from a medium made with oat or corn. The determined LC50 values were 2.81x108 and 3.37x108 conidia/ml when the medium was prepared with 40g of the mentioned ingredients. Meanwhile, LC50 was a high of 8.38x108 and 8.66x108 conidia/ml when the medium was prepared with

Component	Wt (gm/ l)	LC_{50} conidia/ml	slope	r
Barley	20	1.96×10^{8}	0.439	0.983
	40	6.91×10^{7}	0.414	0.993
Corn	20	8.58×10^{8}	0.391	0.995
	40	3.37×10^{8}	0.442	0.994
Wheat	20	5.00×10^{7}	0.441	0.995
	40	2.11×10^{7}	0.502	0.991
Oat	20	8.66×10^{8}	0.381	0.992
	40	2.81×10^{8}	0.414	0.979
Soy bean	20	2.37×10^{8}	0.460	0.988
	40	1.06×10^{8}	0.351	0.991
SDYA	--	3.75×10^{6}	0.64	0.99

Table (2): LC_{50} of red palm weevil adults treated with B. bassiana (B-SA3) conidia harvested from media made with different ingredients in two weights (40 or 20 gm/ liter).

20g of either corn or oat, (Table 2).

The time required to kill 50% (LT50) adults of the red palm weevil treated with 5x109 conidia/ml of B. bassiana (B-SA3) should that conidia cultured on a medium made with 40 or 20 gm of wheat caused the shortest LT50 which was 79.07 and 90.39 hr, respectively, (Table 3). Conidia from the barley medium were slightly superior to that of soy bean when prepared with 40 gm, i.e. 82.41 and 88.51 hr, respectively. Meanwhile, when the medium was prepared with 20 gm of either of these latter mentioned two ingredients; their LT50 values were insignificantly different being 97.95 and 98.17 hr. respectively. The LT50 value of B. bassiana (B-SA3) conidia cultured on oat or corn media was slightly longer as compared with conidia cultured on the other considered media. It is noteworthy, that LT50 value of conidia harvested from the standard SDYA medium was the most superior as it was the lowest recorded, i.e. 73.74 hr (Table 3).

Ingredient	Wt (gm/ l)	LT_{50} (hr)	slope	r
Barley	20	97.95	6.182	0.998
	40	82.41	4.891	0.993
Corn	20	102.92	5.018	0.974
	40	90.36	4.794	0.980
Wheat	20	90.39	6.062	0.979
	40	79.07	6.880	0.998
Oat	20	94.08	2.268	0.997
	40	90.39	4.794	0.980
Soy bean	20	98.17	4.603	0.941
	40	88.51	3.430	0.963
SDYA	-----	73.74	9.14	0.984

Table (3) : LT_{50} (hr) values of red palm weevil adults treated by B. bassiana (B-SA3) conidia at conc. 5 x 10^9 conidia/ ml, harvested from media prepared with 40 and 20 gm/ liter of different ingredients.

Treatment of adults of the red palm weevils with B. bassiana (B-SA3) conidia harvested from different media at a concentration of 5x108 conidia/ml showed that similar to the previous higher considered concentration, conidia cultured on the wheat medium caused the lowest LT50 value. These values were 93.97 and 97.95 hr when the medium was prepared with 40 or 20 gm wheat, respectively (Table 4). The LT50 of conidia collected from a medium made with 40 or 20 gm soy bean was 105.68 and 108.39 hr which was slightly lower than that of conidia produced on barley (i.e. 107.4 and 112.46 hr), prepared in the respective mentioned weights. Also, similar to the previous results, conidia harvested from a medium made with corn or oat were the least virulence against the red palm weevil, (Table 4). Again the virulence of B.bassiana (B-SA3) conidia harvested from the standard SDYA was 76.21 (Table 4).

Ingredient	Wt (gm/ l)	LT_{50} (hr)	slope	r
Barley	20	112.46	4.820	0.952
	40	107.40	4.923	0.999
Corn	20	123.30	3.734	0.977
	40	117.17	3.836	0.988
Wheat	20	97.95	6.182	0.998
	40	93.97	4.699	0.997
Oat	20	119.40	5.791	0.999
	40	117.17	3.836	0.988
Soy bean	20	108.39	6.980	0.992
	40	105.68	3.440	0.970
SDYA	----	76.21	5.6	0.918

Table (4) : LT_{50} (hr) values of red palm weevil adults treated by B. bassiana (B-SA3) conidia at conc. 5x10^8 conidia/ ml harvested from media made with different ingredients

Red palm adult weevils treated with B. bassiana (B-SA3) conidia harvested from tested media and at a concentration of 5x107 conidia/ ml followed the same trend in regard LT50 values as the other two higher tested concentrations but took a longer time. At this concentration the shortest LT50 time was detected with 40 gm of the wheat medium (i.e. 119.67 hr) and the longest time was 195 hr for

conidia produced on 20 gm corn. These periods were relatively comparable to conidia produced on 40 or 20 gm oat as LT50 was 185 and 190 hr , respectively, (Table 5). Conidia of B. bassiana (B-SA3), at the same considered concentration, harvested from the standard SDYA was more virulent to red palm weevil adults, LT50 was 85.7 hr.

The LT50 value could not be calculated for the lower concentrations, (i.e. 5x105 and 5x106 conidia/ml) due to low mortality of treated adult weevils.

5- Mycoses on treated R. *ferugenesis cadavers*:-

As seen in Fig. 5 the highest percentage of visible aerial mycelium (mycosis), on the 7th day post mortem, was detected on cadavers of weevil's treated by B. bassiana (B-SA3) conidia cultured on the standard SDYA medium. These percentages were 100 % at the two highest concentrations of 5x109 and 5x108 conidia/ ml, but declined to 87.5 % at the lower concentrations 5x107 conidia/ ml.

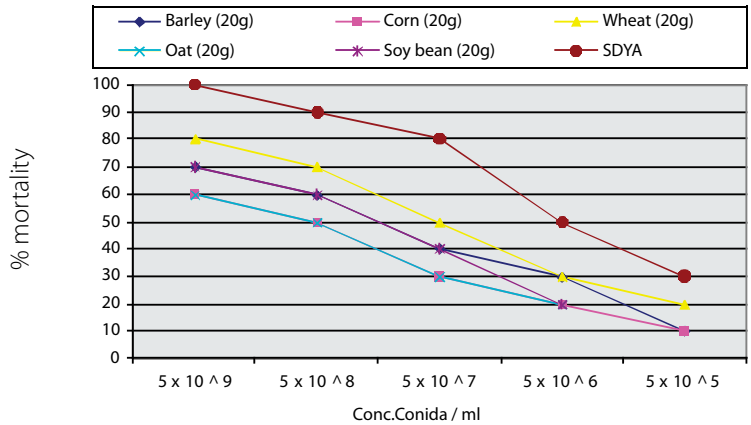


Fig. (3) Accumulated corrected percentage mortality of red palm weevils adults treated with different concentrations of B. bassiana (B-SA3) conidia cultured on media made with 20gm of different ingredients

Of the tested media highest percentage of mycosis was 77.78 % on cadavers of the red palm weevils, by the 7th day following their death, when treated with 5x109 B. bassiana (B-SA3) conidia/ ml harvested from a medium prepared with 40gm of wheat. This was followed by a slightly lower percentage of 75 % by B. bassiana (B-SA3) conidia cultured on barley medium. Also, at this considered concentration, 71.43 % of cadavers of weevils treated with conidia produced on either 40 gm of oat, soy bean or corn exhibited mycosis.

The percentage of mycosis on cadavers of the red palm weevil gradually decreased with a decrease in the administered conidia concentrations as well as when the media was prepared with a lesser weight of the component. The decrease was at a sharper rate in weevil cadavers treated by conidia produced on oat. However, at the lower concentration of 5x105 conidia/ ml, none of the dead treated weevils displayed mycoses.

Use of fungi in practical biological control programs will require production of large amounts of inoculum (Batista- Filho et al., 1988; Jenkins et al.,1998). In the present work, media prepared with available seeds or grains, i.e. wheat, oat, corn, soy bean or barley in agar were considered for the culture of B. bassiana conidia. The SDYA medium was taken as a comparison. From the fore mentioned results, it could be deducted that medium made with 40 gm of any of the considered ingredients were much more resourceful for the production of B. bassiana (B-SA3) than when prepared with the lesser weight of 20 gm. Therefore, the following summation of results present media prepared with

Ingredient	wt (gm/ l)	LT_{50} (hr)	slope	r
Barley	20	170.00	5.915	0.978
	40	158.50	4.723	0.980
Corn	20	195.00	4.312	0.999
	40	175.00	3.340	0.999
Wheat	20	139.00	4.192	0.981
	40	119.67	3.578	0.991
Oat	20	190.00	3.340	0.999
	40	185.00	4.312	0.999
Soy bean	20	160.00	5.915	0.978
	40	138.99	4.190	0.981
SDYA	----	85.7	4.8	0.931

Table (5) : LT^{50} (hr) values of red palm weevil adults treated by B. bassiana (B-SA3) conidia at conc. $5x10^7$ conidia/ ml harvested from media made with different ingredients

Ingredient	Wt (gm/l)	Wt. spores (mg)	N° spores /cm²	% germination.	% decrease than standard medium SDYA			LC 50	LT 50		
					wt	N°. spores	% germination.		5×10⁹	5×10⁸	5×10⁷
Barley	20	0.089	2.9×10^8	95.07	48.80	66.6	3.3	1.96×10^8	97.95	112.46	170.00
	40	0.092	3.1×10^8	96.10	47.12	64.4	2.1	6.91×10^7	82.41	107.40	158.50
Corn	20	0.097	3.1×10^8	96.70	44.25	64.6	1.5	8.58×10^8	102.92	123.30	195.00
	40	0.122	4.3×10^8	97.50	29.80	50.5	0.7	3.37×10^8	90.36	117.17	175.00
Wheat	20	0.138	6.4×10^8	97.30	20.60	26.4	0.9	5.00×10^7	90.39	97.95	139.00
	40	0.171	7.6×10^8	97.60	17.20	12.6	0.6	2.11×10^7	79.07	93.97	119.67
Oat	20	0.127	5.5×10^8	96.50	27.01	36.7	1.7	8.66×10^8	94.08	119.40	190.00
	40	0.176	7.9×10^8	97.60	17.2	9.0	0.6	2.81×10^8	90.39	117.17	185.00
Soy bean	20	0.086	3.3×10^8	94.02	50.57	62.0	4.18	2.37×10^8	98.17	108.39	160.00
	40	0.132	5.5×10^8	94.40	24.10	36.7	3.8	1.06×10^8	85.70	98.17	124.45
SDYA	-------	0.174	8.7×10^8	98.20	----	----------	------	3.75×10^6	73.74	76.21	85.70

Table (6) : Compiled data of the assessment of medium for the production of B. bassiana (B-SA3).

only 40 gm. As depicted in Table 6, the medium prepared with 40 gm wheat was the most efficient for the production of B. bassiana (B-SA3) to be used in a control program against the red palm weevil. This medium produced 17.2 % conidia less in weight and 12.6 % less in number than those produced on the standard SDYA medium. Furthermore, percentage conidia germination was comparable as it was only 0.6% less than the standard SDYA. In the conducted bioassay against the red palm weevil the LC50 value of conidia produced on wheat were 2.11x 107 conidia/ ml, and surpassed that of conidia produced on other components. Also, in this case, LT50 values were the shortest.

The culture medium prepared with 40 gm oat could be taken in second position, as it produced the same weight of B. bassiana (B-SA3) spores as those obtained on SDYA but less with a slight 9 % and a negligible 0.9 % lower number of conidia/cm2 and germination percentage, respectively, than the standard medium. However, the LT50 of conidia produced on this component (and also those cultured on corn medium) exhibited the longest duration recorded as they ranging between 195 - 175 hr.

A medium made with 40 gm corn produced a very low number of B. bassiana (B-SA3) conidia as it was half

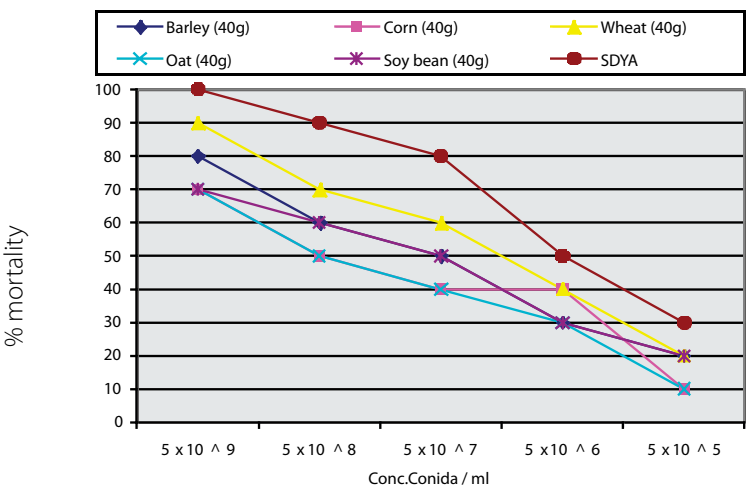


Fig. (4): Accumulated corrected percentage mortality of red palm weevils adults treated with different concentrations of B. bassiana (B-SA3) conidia cultured on media made with 40gm of different ingredients

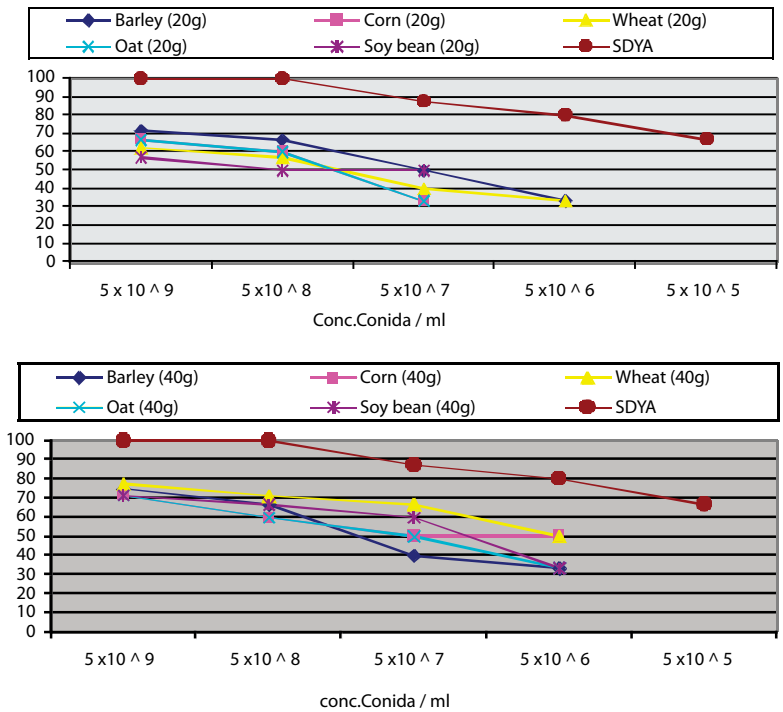


Fig. (5): Percentage of cadavers of red palm weevils exhibiting mycosis following treatment with different concentrations of B. ***bassiana*** (B-SA3) conidia cultured on different media's.

the value produced on SDYA medium (i.e. 50.5 %) but these conidia had a high germination percentage of 97.5 %. The virulence of these conidia against red palm weevil adults was relatively low as detected by the determined LC 50 value which was 3.37x108 conidia/ml.

Of the tested media, that prepared with barley medium produced the lowest number of conidia which was less by 64.4 % than that produced on the SDYA medium. These conidia exhibited a high viability as depicted by germination percentage (96.1 %) but their virulence against red palm weevil adults was low. In contrast LC50 value of conidia produced on soy bean was 1.06 x 108 conidia/ ml when tested on the red palm weevil, which put their virulence in an enhanced position if compared with other tested media. The considered prepared media are not to be under estimated as their conidial yield and viability was relatively reasonable. The lesser infectivity of these conidia as compared to conidia harvested from the standard SDYA medium may be compensated by the addition other additives; further studies are required to confirm this assumption.

Mycosis was apparent on 77.78- 71.43 % of dead treated weevils treated by LC50 values above 5x106 conidia/ ml; it is most significant for the occurrence of mycosis on the cadavers of weevils so as to create an opportunity of spreading the conidia. This calculation would be most important when conducting a control program for the red palm weevil especially that male adults of this insect release an aggregation pheromone (Al-Jahr and Al-Rajeh, 2000), therefore, allowing close contact with other weevils and feasibility of fungal infection spreading between weevils. An experiment conducted by Andrei (2001) showed that B. bassiana conidia in the soil from cadavers of infected Leptinotarsa decemilineata (Say) adults caused high mortality to other insects.

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