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Effect of different grains as solid substrates on sporulation, viability and pathogenicity of *Metarhizium anisopliae* (Metschnikoff) Sorokin

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ABSTRACT: **Influence of different cereal grains as solid substrates on sporulation, viability and pathogenicity of *Metarhizium anisopliae* (Metschnikoff) Sorokin was studied under laboratory condition. Among the six grains (viz. maize, sorghum, fingermillet, sorghum, barley and wheat), sorghum as solid substrate resulted in highest spore (6.36×10^7 conidia ml^{-1}) production with spore viability of 86.6 per cent. The production and viability of conidia increased when the substrates were supplemented with sucrose as compared to conidia produced from substrates alone. Conidia produced from fingermillet showed highest virulence against third instar larvae of *Spodoptera litura* with LC_{50} value of 1.72×10^6 conidia ml^{-1} compared to conidia from other sources. However, conidia produced from SDA medium showed highest spore production, viability and virulence to *S. litura* larvae compared to conidia produced from solid substrates.**

KEY WORDS: *Metarhizium*, pathogenicity, solid substrates fermentation, *Spodoptera* sporulation, viability.

INTRODUCTION

Microorganisms like virus, bacteria, fungi, nematodes and protozoa infecting insect pests and causing epizootics, have proved to be of great potential value for the management of pests. Interest in entomopathogenic fungi as a potential substitutes for chemical insecticides has recently increased owing to the necessity of avoiding adverse effects of chemicals in pest control.

Among the entomogenous fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are important entomogenous fungi that have been found to be pathogenic to agriculturally important orders viz. lepidoptera, coleoptera, hemiptera etc. However, mass production of entomogenous fungi and testing of virulence are important steps in successful utilization of entomogenous fungi. The production of conidia and their viability dependents

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upon the culture medium and carbon source plays important role in determining the quantity and quality of conidia in terms of spore germination and infection. Latch and Fallon (1976) have reported the use of cereals grains for mass production of several entomogenous fungi. Rao (1989) reported that rice grain was suitable for multiplication of *M. anisopliae*. However, Quintela (1994) reported that production of conidia of *M. anisopliae* on coarse grain was significantly greater than whole grain.

In light of above facts, present study was undertaken to assess the influence of different grains as solid substrates on spore production, viability and pathogenicity of *M. anisopliae*.

MATERIALS AND METHODS

To study the effect of different grains as solid substrates on spore production and their viability, six grains namely, sorghum, wheat, barley, soybean, fingermillets and maize were used for the production of *M. anisopliae* conidia. Fifty grams each of sorghum, wheat, barley, soybean, fingermillets and maize grains were soaked in distilled water for 1-2 hours in two Erlenmeyer flasks. The soaked grains were washed with fresh water and two grams of sucrose was added as carbon source in one of the treatments. All the flasks containing media with or without sucrose were autoclaved at 15 lbs psi for 40 minutes. After cooling, each flask were inoculated with five mm discs of *M. anisopliae* (Pantnagar isolate) which were cut from seven day old actively growing culture. Flask containing SDA (Sabourad dextrose agar) medium was also inoculated that served as control. The flasks with different media were incubated at 25 ± 2°C and 95 ± 5 per cent relative humidity for 18 days. There were four replications for each treatment. For conidial count, homogenous conidial suspension was prepared by adding 0.02 per cent Tween 80 as a wetting agent. Then the flasks were shaken thoroughly to prepare proper suspension. The prepared suspension was passed through a double-layered muslin cloth. The conidial counts were made from serially diluted conidial suspensions using improved Neubnauer haemocytometer.

To observe the conidial viability conidial suspensions were prepared. One ml of each fungal suspension was poured in Petri dishe containing very thin layer of SDA media and kept for 16-24 hrs at 25±2°C and 95 per cent relative humidity for germination. The per cent germination of conidia was determined.

To observe the influence of different that were used grains as substrates, on pathogenicity of *M. anisopliae*, the conidial suspensions were prepared in 100 ml distilled water containing 0.02 per cent Tween-80 from the different grains based substrates. From the stock solution, 10^5, 10^6, 10^7, 10^8 and 10^9 conidia ml^{-1} suspensions were prepared. Third instar larvae of *S. litura* were treated with these suspensions and incubated at 25 ± 2°C and 95 per cent relative humidity. There were three replications. The observations on mortality due to mycosis were recorded at 24 hours intervals upto 10 days and data were subjected to probit analysis (Finny, 1964). For calculation of LT_{50} value, mortality at the conidial suspension with 10^7 conidia ml^{-1} was used.

RESULTS AND DISCUSSION

Conidia of *M. anisopliae* produced from different solid substrates, viz. maize, sorghum, soybean, fingermillets, barley and wheat, showed variable response in spore production, viability and pathogenicity.

Effect on spore production

The tested media showed varying response on spore production. Among the substrates tested highest conidial production was observed with sorghum grains as substrates i.e. 6.36×10^7 conidia ml^{-1} which was significantly higher than that produced from soybean (4.46×10^7 conidia ml^{-1}) and maize (4.42×10^7 conidia ml^{-1}) and at par with fingermillet (6.06×10^7 conidia ml^{-1}), wheat (5.52×10^7 conidia ml^{-1}) and barley (5.0×10^7 conidia ml^{-1}). The lowest production of conidia was observed with maize as substrate (4.42×10^7 conidia ml^{-1}) followed by soybean (4.46×10^7 conidia ml^{-1}). The SDA media produced highest conidia (6.86×10^7 conidia ml^{-1}) compared to all the solid substrates

Table 1. Effect of different grains as solid substrates on spore production and viability of *M. anisopliae* (Pantnagar isolate)

Grain media	Spore (x 10^7conidia ml^{-1})		Viability (%)	
	A	B	A	B
Maize	4.42(35.57)	4.76(30.61)	72.4(22.78)	73.53(21.57)
Sorghum	6.36(7.29)	6.85(10.15)	80.6(14.03)	87.83(6.31)
Fingermillets	6.06 (11.66)	6.83(0.44)	86.6(7.63)	88.20(5.02)
Soybean	4.46(34.98)	4.93(28.13)	72.0(23.20)	73.26(21.85)
Barley	5.00(27.11)	5.20(24.19)	77.6(17.23)	78.40(16.67)
Wheat	5.52 (19.5)	5.98(12.83)	79.4(15.31)	80.60(14.0)
SDA	6.86	6.86	93.73	93.73
CD (P = 0.05)SEM ±	1.461.48	1.300.43	4.531.51	3.771.25

Data in parentheses indicated per cent reduction over SDA medium

A= without sucrose

B= with sucrose

(Table 1). Maize, sorghum, fingermillets, soybean, barley and wheat as substrate resulted in 35.6, 7.3, 11.7, 34.9, 27.1 and 19.5 per cent reduction conidia in production, respectively, as compared to conidia produced from SDA medium (Table 1). The production of conidia increased when the substrate were supplemented with sucrose as compared to conidia produced on substrates alone (Figure 1). The highest and lowest conidia production was 6.85 and 4.76 x 10^7 conidia ml^{-1} with sorghum and maize as substrates, respectively. Increase in production of conidia in sucrose-supplemented media was due to extra carbon source which acts as nutrient for the fungus. Magalesh *et al.* (1994) reported that best medium composition for mycillial growth of *M. anisopliae* was four per cent sucrose with one per cent yeast extract. SDA media produced 30.6, 10.2, 0.44, 28.1, 24.2 and 12.8 per cent more conidia compared to maize, sorghum, fingermillet, soybean, barley and wheat grain medium, respectively, when media were supplemented with sucrose as carbon source. However, conidia production in fingermillets supplemented with sucrose was approximately equal to conidia produced on SDA medium (Figure 1).

Effect on viability

Conidia produced on different grains as substrates showed variation in viability. Highest conidial viability was 93.73 percent in conidia produced from SDA medium. However, among the grains used as substrates, highest viability was recorded in conidia produced with fingermillet (86.6 percent) followed by sorghum (80.6 percent) and wheat (79.4 percent). The lowest viability was observed in conidia produced with soybean (72.0 percent) followed by maize (72.4 percent). The maize, sorghum, fingermillets, soybean barley and wheat resulted in 22.8, 14.0, 7.6, 23.2, 17.2 and 15.3 per cent less production of viable conidia, respectively, compared to conidia produced from SDA medium. Viability of conidia produced in different grains as substrates slightly increased when media were supplemented with sucrose (Figure 2). Highest conidial viability was 88.2 per cent in fingermillet that was significantly higher than all the grain based media except sorghum in which viability of conidia was 87.83 per cent. Patel (1997) reported that sorghum was the best substrate for growth and sporulation of *M. anisopliae*. However, viability of conidia was highest in SDA medium compared to

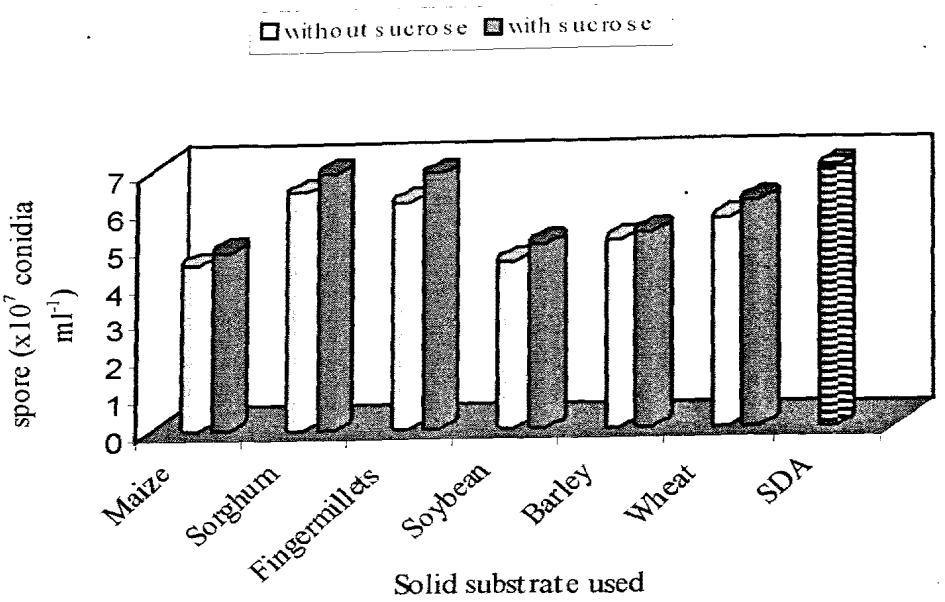

Fig. 1. Effect of different grains as solid substrates on spore production of *M. anisopliae* (Pantnagar isolate)

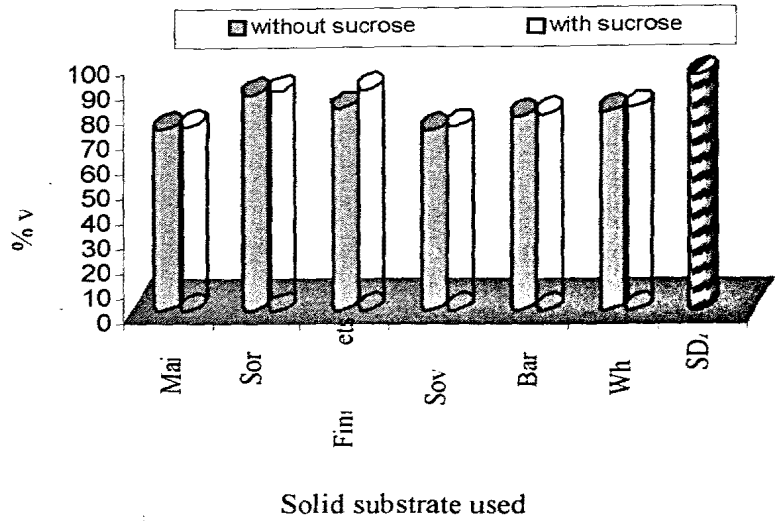

Fig. 2. Effect of different grains as solid substrates on viability *M. anisopliae* (Pantnagar isolate) conidia.

all the grain-based medium. Similar findings have been reported by Pandey and Kanujia (2005) also.

Effect on pathogenicity

Pathogenicity tests of *M. anisopliae* conidia produced on different grains as solid substrates were carried out against third instar larvae of *S. litura*. Panthogenicity tests showed varying degree of virulence to *S. litura* larvae (Table 3 and Figure 3). Conidia produced from fingermillet showed higher virulence to third instar larvae of *S. litura* with the LC_{50} and LT_{50} value of 1.72 x 10^6 conidia ml^{-1} and 159.1 h, respectively, followed by conidia produced on sorghum (3.26 x 10^6 conidia and 162.0 h), barley (6.44 x 10^6 conidia ml^{-1} and 159.7 h). Similar report has been made by Sankaranarayanan, (2000).

Table 2. LC_{50} values of *M. anisopliae* against third instar larvae of *S. litura*

Grain media	χ^2 value	Regression equation Y = a + bx	LC_{50} (x 10^6 conidia ml^{-1})	Fiducial limit (conidia ml^{-1})	Decrease in virulence over SDA media
Barley	0.410	Y = 3.8984 + 0.1617 x	6.445(3.73)	1.894 x 10^8 - 2.192 x 10^5	4.81
Fingermillets	0.031	Y = 4.0117 + 0.1584 x	1.726(1.00)	1.147 x 10^8 - 2.598 x 10^4	1.29
Maize	0.084	Y = 4.1318 + 0.1272 x	6.655 (3.85)	4.769 x 10^8 - 9.288 x 10^4	4.97
Sorghum	0.265	Y = 3.8033 + 0.1837 x	3.264 (1.89)	8.773 x 10^8 - 1.220 x 10^5	2.43
Soybean	0.444	Y = 3.7523 + 0.1815 x	7.422(4.30)	1.433 x 10^8 - 3.845 x 10^5	5.54
Wheat	0.401	Y = 3.8555 + 0.1679 x	6.517 (3.77)	1.685 x 10^8 - 2.519 x 10^5	4.86
SDA	0.213	Y = 4.0342 + 0.1575 x	1.340 (0.78)	1.079 x 10^7 - 1.679 x 10^4	1.00

Data in parentheses indicate relative decrease in virulence over fingermillet

Table 3. LT_{50} values of *M. anisopliae* against third instar larvae of *S. litura*

Grain media	χ^2 value	Regression equation Y = a+ bx	LT_{50}(h)	Fiducial limit (h)
Barley	0.558	Y = 3.4934 + 3.8544 x	159.7	183.8 – 138.8
Fingermillete	0.489	Y = 4.4160 + 4.2764 x	159.1	159.1 – 140.2
Maize	1.197	Y = 3.8633 + 3.9796 x	168.7	195.5 – 145.6
Sorghum	1.939	Y = 4.5867 + 4.3387 x	162.0	184.1 – 142.5
Soybean	1.116	Y = 4.6664 + 4.3372 x	169.3	194.2 – 147.6
Wheat	3.384	Y = 5.2567 + 4.6306 x	164.0	177.6 - 103.2
SDA	4.830	Y = 4.2244 + 4.1996 x	157.2	203.2 - 121.6

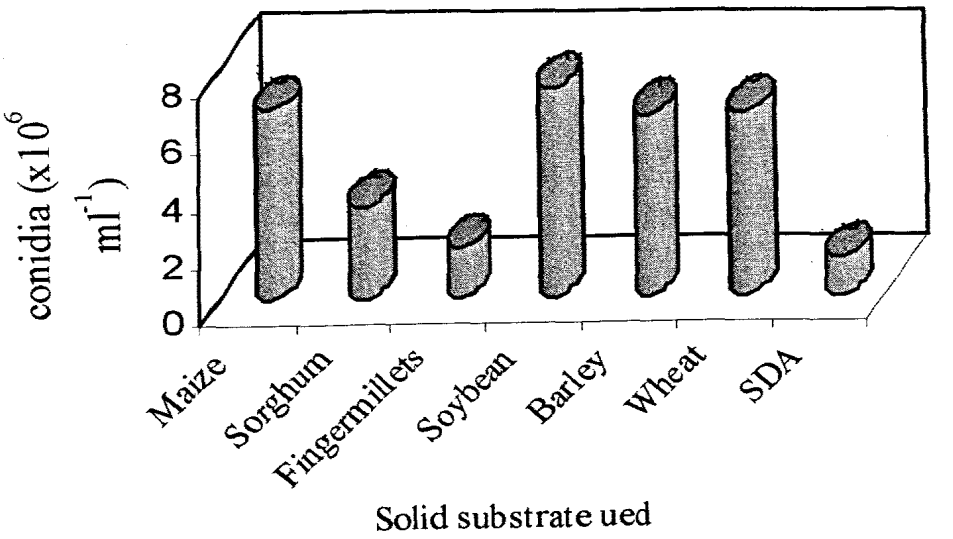

Fig 3. LC_{50} value of *M. anisopilae*, cultured on different grains on solid substrates against third instar larvae of *S. litura*

He found that conidia produced on sorghum had more virulence than conidia produced on rice and wheat. Conidia produced on soybean showed lower virulence to *S. litura* larvae with the LC_{50} and LT_{50} value of 7.42 x 10^6 conidia ml^{-1} and 169.3 h, respectively. Conidia produced on fingermillet showed 1.89, 3.73, 3.77, 3.85 and 4.30 times more virulence to *S. litura* than conidia produced on sorghum, barley, wheat, maize and soybean, respectively (Table 2). However, among all the media tested, conidia produced from SDA media showed highest virulence to *S. litura* larvae with LC_{50} and LT_{50} value of 1.36 x 10^6 conidia ml^{-1} and 157.2 h, respectively, which showed 1.29, 2.43, 4.81, 4.86, 4.39 and 5.54 times more virulence to *S. litura* larvae, on the basis of LC_{50} value, than the virulence of conidia produced on fingermillet, soybean, barley, wheat, maize and soybean, respectively.

On the basis of the alone results, it may concluded that among the different grain as substrates, fingermillet produced highest number of viable conidia with highest virulence against *S. litura* larvae. However, SDA media was superior among all the media.

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