BIOEFFICACY OF *METARHIZIUM ANISOPLIAE* ISOLATES AGAINST TEAK SKELETONISER *PALIGA MACHOERALIS* (LEPIDOPTERA: PYRALIDAE)

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SAPNA-BAI N, REMADEVI OK, SASIDHARAN TO, BALACHANDER M & DHARMARAJAN P. 2013. Bioefficacy of *Metarhizium anisopliae* isolates against teak skeletoniser *Paliga machoeralis* (Lepidoptera: Pyralidae). *Paliga machoeralis* is the most malicious pest of teak responsible for epidemic defoliation of trees in plantations and natural forests. *Metarhizium* spp. have been a long standing model for biological control reported to have great potential for the management of over 200 insect species. The present study was conducted to evaluate the virulence of *M. anisopliae* isolates against *P. machoeralis* in the laboratory. Bioassay of 25 isolates of *M. anisopliae* was carried out using inoculum concentrations ranging from 10^3–10^8 conidia ml^{-1}. The dose-mortality and time-dose-mortality responses for these isolates were determined. Median lethal dose concentration (LC_{50}) values of isolates ranged from 0.11×10^5 to 3417.65×10^5 conidia ml^{-1}. Among the 25 isolates, MIS2, MIS7, MIS1 and MIS3 were found to be more effective with lower LC_{50} values. MIS2 was the most effective isolate with lowest LC_{50} (0.11×10^5 conidia ml^{-1}) followed by MIS7 (0.15×10^5 conidia ml^{-1}). Lowest median lethal time (LT_{50}) of 3.4 days was also recorded for MIS2 followed by MIS7 (3.7 days), MIS1 (4.3 days) and MIS3 (4.9 days) at spore load of 10^7 conidia ml^{-1}. With respect to LC_{50} and LT_{50}, MIS2 proved to be superior over other isolates. The results indicate prospects of isolates MIS2 and MIS7 in developing biopesticide formulation for management of teak skeletoniser.

Keywords: *Tectona grandis*, entomopathogenic fungi, biological control, pathogenicity, pest

SAPNA-BAI N, REMADEVI OK, SASIDHARAN TO, BALACHANDER M & DHARMARAJAN P. 2013. Bioefikasi *Metarhizium anisopliae* menentang ulat perangka daun jati *Paliga machoeralis* (Lepidoptera: Pyralidae). *Paliga machoeralis* merupakan perosak jati yang paling teruk dan menyebabkan epidemik peluruhan daun di ladang serta hutan asli. *Metarhizium* spp. sudah lama diguna untuk kawalan biologi dan mempunyai potensi besar dalam pengurusan lebih 200 spesies serangga. Kajian ini dijalankan untuk menilai kemudaratan isolat *M. anisopliae* terhadap *P. machoeralis* di makmal. Bioasai 25 isolat *M. anisopliae* dijalankan menggunakan kepekatan inokulum 10^3–10^8 konidium ml^{-1}. Kematian isolat berdasarkan dos serta masa ditentukan. Kepekatan maut purata (LC_{50}) isolat berjulat antara 0.11×10^5 konidium ml^{-1} hingga 3417.65×10^5 konidium ml^{-1}. Antara 25 isolat yang dikaji, MIS2, MIS7, MIS1 dan MIS3 didapati lebih berkesan dengan nilai LC_{50} yang lebih rendah. MIS2 merupakan isolat yang paling berkesan dengan nilai LC_{50} yang terendah iaitu 0.11×10^5 konidium ml^{-1}. Ini diikuti oleh MIS7 (0.15×10^5 konidium ml^{-1}). Nilai masa maut purata (LT_{50}) yang terendah iaitu 3.4 hari dicerap untuk MIS2 diikuti oleh MIS7 (3.7 hari), MIS1 (4.3 hari) dan MIS3 (4.9 hari) pada kepekatan 10^7 konidium ml^{-1}. Dari segi LC_{50} serta LT_{50}, MIS2 nyata lebih berkesan berbanding dengan isolat lain. Keputusan menunjukkan potensi isolat MIS2 dan MIS7 dalam penghasilan biopestisid untuk pengurusan ulat perangka daun jati.

INTRODUCTION

The teak skeletoniser *Paliga machoeralis* is the most pernicious pest of teak responsible for epidemic defoliation in nurseries, plantations and natural forests throughout South Asia and some parts of South-East Asia (Kulkarni et al. 2011). Outbreaks of this pest occur in most years with exceptionally heavy build-up in some years. Although the insect is present throughout the year, outbreaks develop towards the end of the growing season before normal leaf shedding (Nair 2001). Larvae of this insect feed only on fleshy leaf tissues, leaving all veins intact resulting

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in both qualitative and quantitative losses in timber production (Roychoudhury & Dadwal 2010). Damage varies from almost negligible to as much as half of the total annual volume increment. Along with *Hyblaea puera*, it causes losses amounting to 65% in plantations and 55% in seedlings in nurseries (Kulkarni et al. 2011).

In India, teak pests are reported to be infected by several entomopathogens. The fungus *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) is one of the most common entomopathogen with worldwide distribution as a component of natural soil flora (Scholte et al. 2004). *Metarhizium* has been widely researched and has been reported to have great potential for use as a biological control agent for the management of various insect pests (Inglis et al. 2001, Liu et al. 2003, Khashaveh et al. 2008). It is particularly promising because of its wide geographical range and vast spectrum of infectivity to a wide range of insect pests (Zimmermann 2007).

This study was conducted to evaluate the susceptibility of *P. machoeralis* to different isolates of the entomopathogenic fungus *M. anisopliae* under laboratory conditions. Bioefficacy of 25 isolates of *M. anisopliae* was assessed to establish their virulence against *P. machoeralis* with the objective of identifying potential strains.

MATERIALS AND METHODS

Insect culture

Healthy larvae of *P. machoeralis* collected from the field (Figure 1) were reared in the laboratory and allowed to pupate and develop into adults. Male and female moths were released into glass bottles covered with muslin cloth for mating and egg laying. Dilute sucrose solution (10%) was provided on cotton balls as food. The muslin cloths with eggs were surface sterilised with 1% sodium hypochlorite for 15 min and washed in sterile distilled water for 10 min and placed over blotting paper for drying. They were then covered with tender leaves of teak and transferred to glass bottles for hatching of eggs. Larvae established on tender leaves were transferred with fine camel hair brush to plastic containers (14 cm diameter, 6 cm height) containing fresh leaves. The petiole of leaf was wrapped in a layer of moist tissue paper and sealed with parafilm to prevent wilting. Fresh leaves were provided once every 2 days.

Metarhizium culture

Among the 25 fungal isolates (MIS1 to MIS25) used in this study, 16 were isolated either from soil or from infected insects and 9 procured from different institutions. Soil samples were collected from a depth of 30 cm from different study areas. Galleria bait method was used to isolate the fungi from soil samples. After removing roots and gravel, soil samples were sifted through a 5-mm sieve. Thereafter, plastic containers (8 cm diameter, 10 cm height) were filled with 100 g soil and 10 late instar larvae of *Galleria mellonella* were introduced. The lids were punched for air holes. Larvae were incubated at 20 °C in dark conditions. During the first five days, the containers were turned once daily to make bait insects penetrate as much soil as possible. After 7–10 days, containers were examined every day and dead larvae were collected. Cadavers thus obtained, as well as those collected from field were surface-sterilised by dipping consecutively in 70% ethyl alcohol, 1% sodium hypochlorite and finally sterile distilled water, each for 3 min. The larvae were dissected and placed on Veen's medium and incubated at 28 ± 1 °C and 90% relative humidity to facilitate growth and sporulation of fungus. Slant cultures were prepared from a single colony and stored at -20 °C until use.

Inoculum preparation

Culture plates of each isolate were prepared by spreading 200 µl of conidial suspension (10^7 conidia ml^{-1}) onto dextrose agar enriched with yeast extract medium. Plates were incubated in the dark at 28 ± 1 °C for 14 days to maximise spore production. To harvest spores, each plate was flooded with 10 ml 0.05% Tween 80 in sterile distilled water and the conidia were dislodged into suspension with a glass rod. The suspension was filtered through a double layer sterile cheese cloth and centrifuged at 1700 rpm for 15 min. The supernatant was discarded and the conidia resuspended in 5 ml sterile distilled water. This stock of spore suspension was stored at 4 °C for 24 hours until spore viability was determined. Only cultures with > 90% viability were used. Counts of conidia were made from the stock suspension using an improved haemocytometer. Spore suspensions containing 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia ml^{-1} sterile distilled water with 0.05% Tween 80 were prepared from the stock for bioassay.

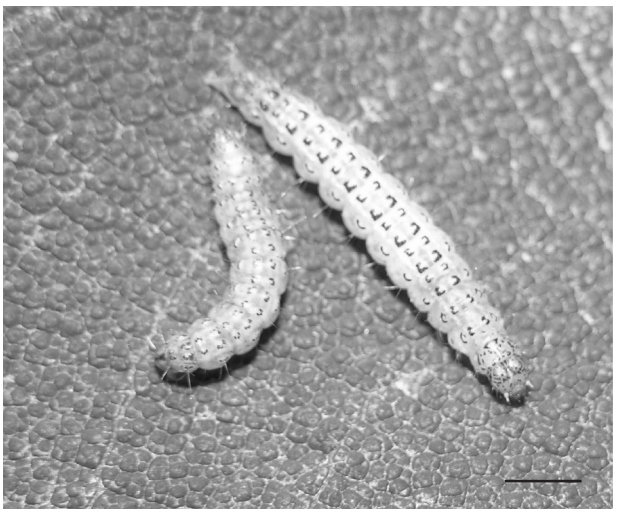

Figure 1 Healthy larvae of *Paliga machoeralis*; scale bar = 2.5 mm

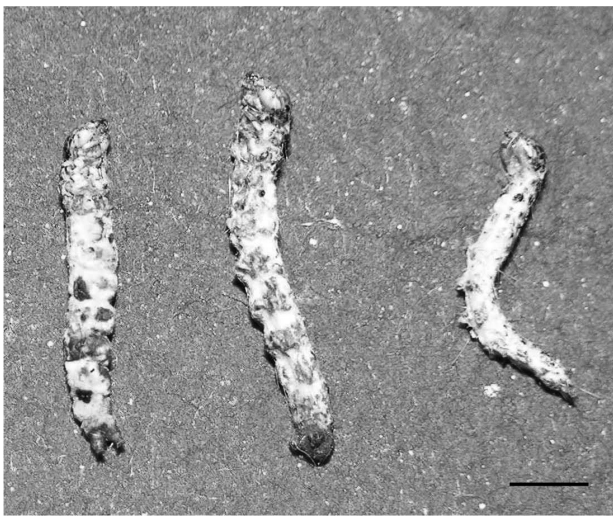

Figure 2 Mycosed cadavers of *Paliga machoeralis*; scale bar = 2.5 mm

Bioassay

Bioassay of all the 25 *M. anisopliae* isolates was carried out against *P. machoeralis* using inoculum concentrations ranging from 10^3–10^8 conidia ml^{-1} to determine the multiple- and time-dose-mortality responses. A total of 30 second instar larvae of *P. machoeralis* were placed separately in sterile 20 ml vials containing 10 ml fungal suspension. The vial was capped and inverted five times over a 5 s period to ensure that the insects were completely drenched with fungal suspension. The suspension with insects was filtered through a tea strainer (6 cm diameter). For controls, insects were treated with 0.05% Tween 80. Treated and untreated (control) larvae were transferred with fine camel hair brush to separate plastic containers (14 cm diameter, 6 cm height) containing fresh leaves as food. To prevent wilting, the petiole of the leaf was wrapped in a layer of moist tissue paper and sealed with parafilm. A vented lid with mesh screen was used to close the plastic containers which were incubated at 26 ± 1 °C, 90% relative humidity and 12:12 light:day. Fresh leaves were provided every 2 days. Four replications were maintained for each concentration of a single isolate. Mortality of larvae was recorded every 24 hours for 8 days after exposure. Dead larvae were counted and removed each day to prevent horizontal contamination. The dead larvae from each treatment were incubated in moist conditions to determine if death resulted from mycosis (Figure 2).

Data analysis

Mortality observed in control experiments was used to correct mortality in the treated groups using the formula by Abbott (1925). Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) for the 25 isolates were estimated by Probit analysis (Finney 1971). Probit analysis was carried out using SPSS software program version 12.

RESULTS AND DISCUSSION

The LC_{50} of the isolates ranged from 0.11×10^5 to 3417.65×10^5 conidia ml^{-1} (Table 1). MIS2 was the most effective isolate with lowest LC_{50} (0.11×10^5 conidia ml^{-1}) followed by MIS7 (0.15×10^5 conidia ml^{-1}), MIS1 (0.61×10^5 conidia ml^{-1}) and MIS3 (4.72×10^5 conidia ml^{-1}). MIS15 was the least effective isolate with highest LC_{50} of 3417.65×10^5 conidia ml^{-1}. At spore load of 10^7 conidia ml^{-1}, lowest LT_{50} was recorded for MIS2 (3.4 days) followed by MIS7 (3.7 days), MIS1 (4.3 days) and MIS3 (4.9 days) (Table 2). The isolate MIS2 took 4.1, 5.0 and 5.7 days to kill 50% population at 10^6, 10^5 and 10^4 conidia ml^{-1} respectively. LT_{50} values of the isolate MIS7 at 10^6, 10^5 and 10^4 conidia ml^{-1} were 4.1, 4.9 and 6.3 days respectively. The LT_{50} values varied from 4.3 to 6.2 days for MIS1 and 4.9 to 7.6 days for MIS3 depending on the spore load. With respect to LC_{50} and LT_{50}, MIS2 proved to be superior over other isolates against *P. machoeralis*.

Table 1 Dose-mortality response (LC_{50}) of *Metarhizium anisopliae* isolates to *Paliga machoeralis*

Rank	Isolate	LC_{50} ($\times 10^5$)	Fiducial limit		Slope ± SE	χ^2	p
			Lower ($\times 10^5$)	Upper ($\times 10^5$)			
1	MIS2	0.11	0.00008	0.73239	1.6 ± 0.7	0.197	0.906
2	MIS7	0.15	0.00644	0.57949	2.4 ± 0.8	0.035	0.983
3	MIS1	0.61	0.00094	4.59803	1.5 ± 0.7	0.272	0.873
4	MIS3	4.72	0.66243	56.64603	2.2 ± 0.7	0.004	0.998
5	MIS18	10.37	1.91863	248.10526	2.4 ± 0.7	0.017	0.991
6	MIS20	13.86	3.05288	257.88065	2.7 ± 0.7	0.293	0.864
7	MIS10	22.20	7.00368	161.08842	3.9 ± 0.9	0.464	0.793
8	MIS23	26.56	8.20376	220.22594	3.9 ± 0.9	0.474	0.789
9	MIS13	32.33	5.55009	9348.88804	2.4 ± 0.7	0.191	0.909
10	MIS24	46.16	14.38931	485.15540	4.3 ± 1.0	1.659	0.436
11	MIS19	46.34	-	-	1.7 ± 0.7	0.742	0.690
12	MIS11	46.64	5.84365	7686.93360	2.1 ± 0.7	0.792	0.673
13	MIS8	48.67	11.89630	1447.01472	3.4 ± 0.8	1.311	0.519
14	MIS5	60.12	13.93985	2606.62338	3.4 ± 0.9	0.692	0.708
15	MIS4	77.64	18.22976	3334.50181	3.7 ± 0.9	0.189	0.910
16	MIS9	93.05	19.35935	9189.49022	3.5 ± 0.9	1.075	0.584
17	MIS12	116.20	20.57793	43789.67150	3.2 ± 0.9	0.606	0.739
18	MIS22	119.93	24.90833	11820.25079	3.7 ± 0.9	0.172	0.918
19	MIS25	123.14	33.27831	3809.16306	4.8 ± 1.2	0.612	0.736
20	MIS17	182.48	41.04570	18122.41542	4.5 ± 1.1	0.800	0.670
21	MIS16	218.97	24.33625	4344.42244	2.7 ± 0.8	0.024	0.988
22	MIS6	344.54	41.35598	8152.21599	3.3 ± 0.9	0.093	0.955
23	MIS21	568.34	88.30976	4565.70851	4.5 ± 1.4	0.293	0.864
24	MIS14	1681.72	-	-	6.5 ± 2.8	0.216	0.898
25	MIS15	3417.65	123.46164	3.507090E+31	3.1 ± 1.0	0.113	0.945

SE = standard error

A judicious strategy is required for the management of *P. machoeralis* in forest nurseries, where management related practices have been limited to the use of chemical insecticides (Joshi et al. 2001). Work on use of *Metarhizium* fungus for control of *P. machoeralis* is scarce in India. Most of the reported studies involved the use of *Beauveria bassiana, Bacillus thuringiensis* and plant products. Infection of *B. bassiana* on larvae of *Eutectona machaeralis* was recorded for the first time in Kunsi village, Shimoga, Karnataka (Patil & Thontadarya 1981). Laboratory studies to determine the efficacy of *Beauveria brongniartii* (10^4 to 10^8 conidia ml^{-1}) against teak skeletoniser, *Eutectona machaeralis* was conducted by Juliya et al. (2009). They reported an LC_{50} value of 9.40×10^5 conidia ml^{-1} and LT_{50} of 85.03 hours at 10^8 conidia ml^{-1} concentration. LC_{50} value increased with growth and development of larvae (Roychoudhury & Dadwal 2010). Commercial preparations of *B. thuringiensis* have been shown to be effective against *E. machaeralis* under laboratory conditions (Misra & Singh 1993, Roychoudhury et al. 1994).

This study highlights the prospects of *Metarhizium* spp. for use in biological control of teak skeletoniser. Isolates MIS2 and MIS7 could be exploited for commercial development of biopesticide for management of this insect pest. Further studies to ascertain the efficacy of the isolates in the field, effects of environmental conditions on growth and sporulation of isolates and compatibility of the isolates with different oils will pave way for developing effective products for addressing the skeletoniser problem in teak plantations.

Table 2 Time-dose-mortality response of *Metarhizium anisopliae* isolates to *Paliga machoeralis*

Isolate	Conidia concentration	LT_{50}	Fiducial limit		Slope ± SE	χ^2	p
			Lower	Upper			
MIS 1	1×10^4	6.2	5.4	9.2	4.7 ± 1.2	1.46	0.83
	1×10^5	5.9	5.1	8.0	4.4 ± 1.0	0.13	0.99
	1×10^6	4.7	4.0	5.7	2.9 ± 0.5	0.29	0.99
	1×10^7	4.3	3.7	5.4	2.3 ± 0.4	1.74	0.78
MIS 2	1×10^4	5.7	4.7	8.7	2.7 ± 0.6	2.27	0.68
	1×10^5	5.0	4.1	7.0	2.2 ± 0.4	1.40	0.84
	1×10^6	4.1	3.5	5.0	2.2 ± 0.4	0.88	0.92
	1×10^7	3.4	2.8	4.1	1.6 ± 0.3	4.20	0.37
MIS 3	1×10^4	7.6	6.0	28.7	4.6 ± 1.3	0.93	0.92
	1×10^5	6.4	5.6	10.3	5.2 ± 1.4	0.40	0.98
	1×10^6	5.7	4.8	7.9	3.4 ± 0.7	1.08	0.89
	1×10^7	4.9	4.2	6.0	3.2 ± 0.6	0.48	0.97
MIS 4	1×10^4	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^5	8.1	-	-	7.2 ± 3.5	0.15	0.99
	1×10^6	7.4	5.9	18.7	3.9 ± 1.0	0.27	0.99
	1×10^7	6.0	5.1	8.9	3.7 ± 0.8	0.71	0.95
MIS 5	1×10^4	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^5	7.5	6.2	45.2	7.0 ± 2.8	0.47	0.97
	1×10^6	6.4	5.4	10.3	3.9 ± 0.9	0.55	0.96
	1×10^7	6.0	5.1	8.9	3.7 ± 0.8	0.71	0.95
MIS 6	1×10^4	8.1	-	-	7.2 ± 3.5	0.15	0.99
	1×10^5	7.5	-	-	14.3 ± 10.7	0.04	1.00
	1×10^6	7.5	6.1	37.0	5.3 ± 1.7	0.22	0.99
	1×10^7	6.9	5.7	13.5	4.2 ± 1.1	0.49	0.97
MIS 7	1×10^4	6.3	5.6	9.3	6.1 ± 1.7	0.23	0.99
	1×10^5	4.9	4.4	5.6	4.8 ± 1.0	0.35	0.98
	1×10^6	4.1	3.6	4.7	3.4 ± 0.6	0.35	0.98
	1×10^7	3.7	3.3	4.1	3.7 ± 0.6	0.77	0.94
MIS 8	1×10^4	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^5	7.5	6.2	45.2	7.0 ± 2.8	0.47	0.97
	1×10^6	6.4	5.3	10.5	3.6 ± 0.8	0.68	0.95
	1×10^7	6.1	5.2	9.3	3.8 ± 0.8	0.85	0.93
MIS 9	1×10^4	8.1	-	-	7.2 ± 3.5	0.15	0.99
	1×10^5	7.5	-	-	14.3 ± 10.7	0.04	1.00
	1×10^6	6.5	5.6	11.0	5.3 ± 1.4	0.43	0.98
	1×10^7	6.3	5.4	9.5	4.4 ± 1.0	0.39	0.98
MIS 10	1×10^4	7.5	-	-	14.3 ± 10.7	0.04	1.00
	1×10^5	7.4	6.2	40.0	7.3 ± 3.0	0.53	0.97
	1×10^6	6.6	5.8	12.2	6.5 ± 2.0	1.02	0.90
	1×10^7	5.2	4.8	5.9	6.0 ± 1.3	0.81	0.93
MIS 11	1×10^4	9.1	6.5	20.1	4.5 ± 1.6	0.89	0.92
	1×10^5	6.8	5.8	14.0	5.9 ± 1.8	0.14	0.99
	1×10^6	6.2	5.4	8.8	5.6 ± 1.5	0.30	0.99
	1×10^7	5.9	5.3	7.4	6.2 ± 1.5	0.30	0.98
MIS 12	1×10^4	20.9	-	-	3.6 ± 1.4	0.93	0.92
	1×10^5	8.4	6.4	13.7	5.1 ± 1.8	0.25	0.99
	1×10^6	6.8	5.7	13.1	5.1 ± 1.4	0.48	0.97
	1×10^7	6.3	5.3	9.8	3.9 ± 0.9	0.34	0.98
MIS 13	1×10^4	12.0	7.2	69.5	3.3 ± 1.0	0.80	0.93
	1×10^5	7.0	5.8	16.0	4.7 ± 1.3	0.95	0.91

(continued)

Table 2 (continued)

Isolate	Conidia concentration	LT_{50}	Fiducial limit		Slope ± SE	χ^2	p
			Lower	Upper			
	1×10^7	5.6	5.1	6.8	5.8 ± 1.4	0.58	0.96
MIS 14	1×10^4	-	-	-	-	-	-
	1×10^5	-	-	-	-	-	-
	1×10^6	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^7	9.5	-	-	4.6 ± 1.7	0.37	0.98
MIS 15	1×10^4	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^5	9.3	-	-	5.6 ± 2.6	1.26	0.86
	1×10^6	8.1	-	-	6.5 ± 2.8	0.76	0.94
	1×10^7	8.0	6.2	38.6	3.7 ± 1.0	0.44	0.97
MIS 16	1×10^4	9.3	-	-	5.6 ± 2.6	1.26	0.86
	1×10^5	8.6	6.4	178.8	4.2 ± 1.3	1.03	0.90
	1×10^6	7.9	6.0	27.5	3.8 ± 1.0	0.21	0.99
	1×10^7	6.3	5.4	9.5	4.4 ± 1.0	0.39	0.98
MIS 17	1×10^4	-	-	-	-	-	-
	1×10^5	7.4	6.2	40.0	7.3 ± 3.0	0.53	0.97
	1×10^6	7.0	-	-	15.7 ± 13.9	0.06	0.99
	1×10^7	6.4	5.6	10.0	6.3 ± 1.8	0.10	0.99
MIS 18	1×10^4	8.6	6.4	178.8	4.2 ± 1.3	1.03	0.90
	1×10^5	6.9	5.7	14.1	4.6 ± 1.2	1.26	0.86
	1×10^6	5.9	4.9	9.0	2.9 ± 0.6	0.32	0.98
	1×10^7	5.1	4.3	6.9	2.5 ± 0.5	0.91	0.92
MIS 19	1×10^4	13.2	7.4	15.2	2.8 ± 0.7	1.71	0.78
	1×10^5	6.9	5.2	14.9	2.3 ± 0.4	0.91	0.92
	1×10^6	6.4	5.2	11.4	3.0 ± 0.6	1.34	0.85
	1×10^7	5.9	5.1	8.0	4.4 ± 1.0	0.13	0.99
MIS 20	1×10^4	9.5	-	-	4.6 ± 1.7	0.37	0.98
	1×10^4	6.7	5.7	12.0	5.0 ± 1.0	0.62	0.96
	1×10^5	6.3	5.3	10.3	3.5 ± 0.8	0.52	0.97
	1×10^6	5.2	4.4	6.7	3.1 ± 0.6	0.67	0.95
MIS 21	1×10^4	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^5	11.5	-	-	5.6 ± 3.4	0.66	0.95
	1×10^6	8.1	-	-	6.5 ± 2.8	0.76	0.94
	1×10^7	7.5	6.1	28.2	5.0 ± 1.5	1.37	0.84
MIS 22	1×10^4	7.5	-	-	14.3 ± 10.7	0.04	1.00
	1×10^5	7.2	6.1	72.7	7.1 ± 2.7	0.35	0.98
	1×10^6	6.7	-	-	15.7 ± 10.9	0.11	0.99
	1×10^7	5.8	5.3	7.2	7.1 ± 1.8	0.38	0.98
MIS 23	1×10^4	7.5	-	-	14.3 ± 10.7	0.04	1.00
	1×10^5	6.6	-	-	15.6 ± 9.2	0.19	0.99
	1×10^6	6.3	5.6	9.3	6.1 ± 1.7	0.23	0.99
	1×10^7	5.4	4.9	6.3	6.1 ± 1.4	0.54	0.96
MIS 24	1×10^4	-	-	-	-	-	-
	1×10^5	6.8	5.8	14.0	5.9 ± 1.8	0.14	0.99
	1×10^6	6.6	-	-	15.6 ± 9.2	0.19	0.99
	1×10^7	5.7	5.0	7.3	4.7 ± 1.0	0.13	0.99
MIS 25	1×10^4	-	-	-	-	-	-
	1×10^5	7.2	6.1	72.7	7.1 ± 2.7	0.35	0.98
	1×10^6	7.0	-	-	15.7 ± 13.9	0.06	0.99
	1×10^7	6.0	5.5	7.4	8.8 ± 2.5	1.26	0.86

SE = standard error

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