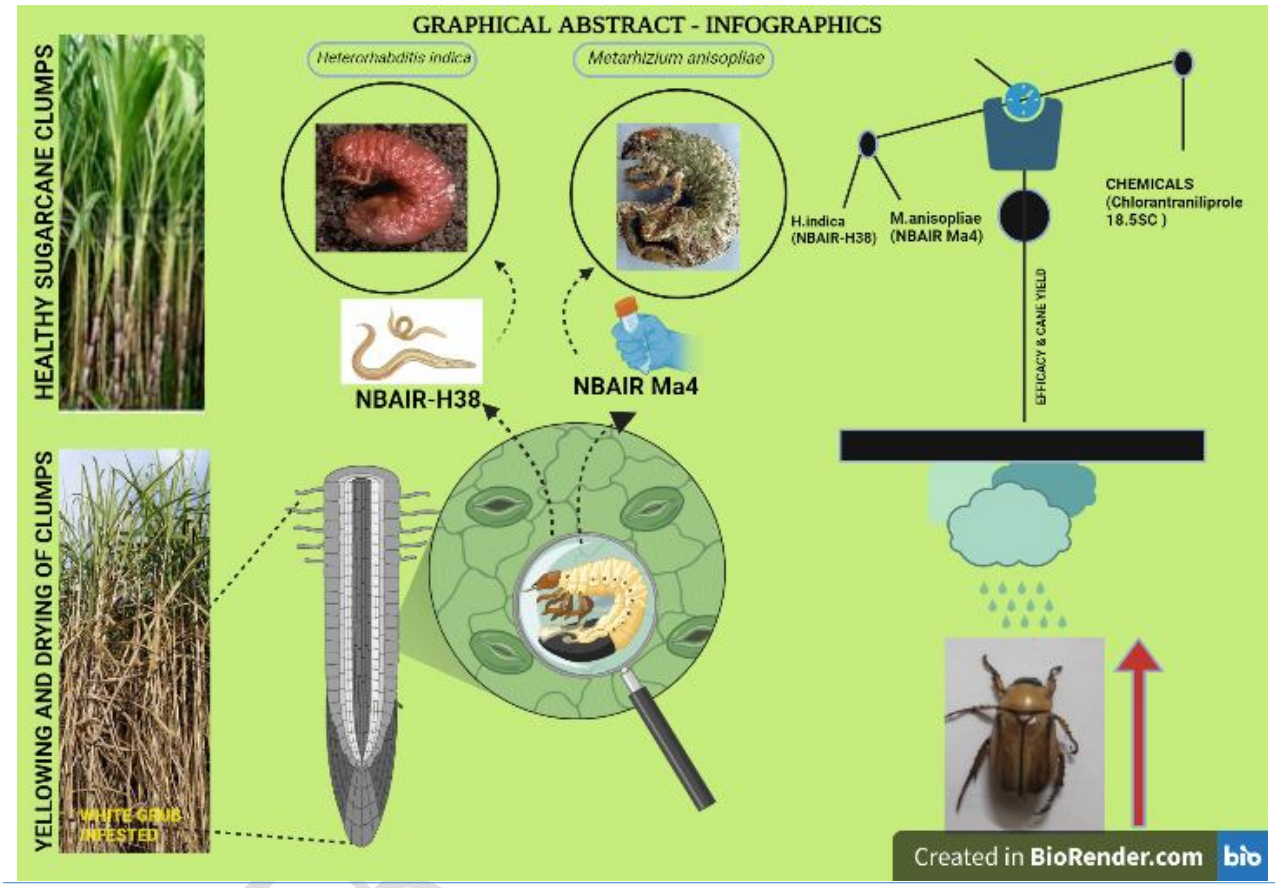
Seasonal incidence and eco friendly management ofwhite grub in sugarcane using entomopathogenic nematode and entomopathogenic fungus in Andhra Pradesh, India

ABSTRACT

High rainfall with increase in humidity (68% to 75%) along with a decrease in temperature (3 to 5 ℃ less than usual) during June, July, and August months favor the emergence of white grub beetles, its egg laying which in turn resulted in severe sugarcane clump damage by white grubsin July, August and September months. Seasonal incidence of white grub in sugarcane growing areas in Andhra Pradesh state showed that damage of sugarcane clumps caused by white grubs infestation was significantly high in march ratoon compared to February ratoon crop; January plant crop and low in January seedling plant crop. Grub damage recorded high in 4 months age sugarcane ratoon crop and low in 6 months and 7 months age sugarcane plant crop. Field efficacy of entomopathogenic nematode and entomopathogenic fungus in the management of white grub, *Holotrichia consanguinea* in sugarcane was investigated and promoted biocontrol in white grub endemic areas during 2017-18 to 2019-20. Entomopathogenic nematode (*Heterorhabditis indica* NBAII-H38), entomopathogenic fungi (*Metarhizium anisopliae* (NBAIR Ma4)along with a chemical Chlorantraniliprole were evaluated through soil application. The results indicated that *Heterorhabditis indica* NBAII-H38 (12 kg *a.i./*ha) soil application two times at monthly interval resulted in higher reduction of white grub damage. *Metarhizium anisopliae*NBAIR Ma4 (2.5 kg a.i./ha) treated plots caused higher percentage reduction of white grub than chlorantraniliprole treatment. Entomopathogenic nematode, *Heterorhabditis indica* and entomopathogenic fungus, *Metarhizium anisopliae* were found effective than the insecticidal application in the management of sugarcane white grub. Cost benefit of

biocontrol agents, *Metarhizium anisopliae* and *H. indica* was proved superior to insecticidal application.

Keywords: Entomopathogenic nematode, *Heterorhabditis indica*, Entomopathogenic fungus, *Metarhizium anisopliae*, White grubs, Sugarcane



INTRODUCTION

Sugarcane is one of India's major commercial crops. Sugarcane is primarily cultivated in several districts of Andhra Pradesh *viz.* Visakhapatnam, West Godavari, East Godavari, Chittoor, Krishna, Vizianagaram, Srikakulam, and Nellore districts, covering 2.40 lakh hectares and producing 136 lakhstons of sugarcane [1]. White grubs are the most obliterating soil pests, causing damage to a wide range of crops. Paramount levels of root damage to India's sugarcane crop are caused by the white grub *Holotrichia consanguinea*, Blanch (Coleoptera: Scarabaeidae). Factors like continuous cultivation of sugarcane also aid in escalating white grub damage .Under severe infestation, which

results in drying and dying of sugarcane clumps as well canes result in complete yield loss (2).

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sugarcane clumps as well mature canes result in complete yield loss [2]. DueDue to its subterranean dwelling nature, predicting the occurrence of white grubs is difficult as well challenging. The emergence, mobility, and distribution of white grub adults are influenced by weather variables like as rainfall, temperature, humidity, and wind velocity [3]. The emergence of the adult of the Indian *Holotrichia* species coincides with the onset of monsoon and is delayed until late in the season.

Apart from feeding on the neem tree foliage, the white grub adults also lay eggs in sugarcane fields. The adults feed on the foliage of the host trees like neem. Third instar grubs feed voraciously on sugarcane roots for three to four months causingcasing yellowing and drying. Sampling techniques for monitoring white grub populations include counting the grubs and formulating indices of population intensity based on plant damage. Several management tactics were used to manage white grubs, including the Argo Ecosystem Analysis approach along with Integrated Pest Management [4-5]. The treatment of white grub is mostly based on insecticide application, which is laborious and expensive as well as polluting the environment. Hence sorting to techniques that which naturally infect white grubs includes the usage of entomopathogens such as fungi (*Beauveria* and *Metarhizium*), bacteria and nematodes which are in vogue from ages old. Entomopathogenic nematodes are opted as an alternative management strategy to curb the white grub menace as they are eco-friendly and cost-effective biological agents [6]. Out of which *Heterorhabditis indica* was found to be encroaching inroads against white grub by Karun akar *et al*.[7]. in roads against white grub by Karunakar *et al* [7]. Keeping the above points in view a study was formulated to investigate the potency of entomopathogenic

nematodes and entomopathogenic fungus against the white grub infesting sugarcane in endemic areas of Andhra Pradesh.

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Andhra Pradesh.

MATERIALS AND METHODS

Preliminary survey was conducted in the sugarcane ecosystems of coastal AndhraPradesh with the help of sugar factory field staff during the month of July, 2017-18 to assess the damage caused by white grub. Observations were undertaken to study the extent of damage cause by white grub in sugarcane plant crop and ratoon crop in four villages viz., Chebrolu, Rothulpudi, P.Champavaram, Boorampalem of East Godavari district, Andhra Pradesh. Observations on number of sugarcane clumps damaged by white grubs and number of grubs per 10-meter row were recorded in sugarcane fields planted or ratooned during the months of January to march month. The presence of grubs per ten-mete row at eight different places at random in the field of sugarcane were used for the estimation of the white grub population. The correlation between white grub population and damaged sugarcane clumps was calculated.

During 2017-18 to 2019-20, field experiments were conducted in white grub endemic areas of Navabharath ventures (Sugar division) operational area, Samalkota, East Godavari dist., Andhra Pradesh to assess the efficacy of entomopathogenic nematode and entomopathogenic fungus in sugarcane. The field trials were set up in a 5-hectare area comprising four treatments, as listed in table 3,4 &,& 5 in a Randomized Block Design (RDB). Each treatment was replicated eight times in a 5-hectare region at random. Ratoon Sugarcane crop was planted in 2017 and was reaped in the year 2018. Likewise in 2018 and 2019, a second -year crop and third year crop were planted and

was harvested in 2019 and 2020. Treatments were applied in furrows after observing white grub incidence after the onset of monsoon season in July during 2017-18, 2018-19 and 2019-20. Plant damage caused by the white grub was observed and noted at monthly intervals until harvest for three rows of ten meters in each treatment. At harvest, the yield of the cane was measured.

M. anisopliae NBAIR-Ma4 was cultured in Sabouraud's Dextrose Yeast Extract Broth (SDYB) (Dextrose 20 g, Mycological peptone 10 g, yeast extract 5 g in 1L distilled water) and then blended with talcum powder at 2% (20 g of pellet in 1 kg talc). *M. anisopliae* NBAIR-Ma4 talc formulations containing 1.0 $x10^8$ were homogenized with FYM @ 2.5 kg Ma4 in 250 kg FYM. The fungus-enriched farmyard manure was employed for the tests after a 15-day incubation period. The experiment used a wettable powder formulation of the entomopathogenic nematode *Heterorhabditis indica* (NBAII H38) procured from NBAIR, Bangalore.

After the commencement of monsoon rains, *M. anisopliae* enhanced FYM was applied to the soil in sugarcane furrows at a rate of 250 kg/ha. Every year, the first application in july, and the second in the month of august were administered. *H. indica*, an entomopathogenic nematode, was applied twice in July and September at a rate of 12 kg ha^{-1} in 150 kg damp sand ha^{-1}. In July and August, a chemical insecticide, Chlorantraniliprole 18.5SC @ 300 ml a.i ha^{-1}, was applied to the soil twice at one-month intervals, with an untreated control plot kept for comparison.

At monthly intervals until harvest, observations on the number of plants injured and the frequency of white grubs per 10 meter rows were made. The recorded cane yield was statistically examined to derive inferences, and a cost-benefit ratio was computed.

Percentage reduction of the grubs = Number of white grubs in control – Number of white grubs in treatment/ Number of white grubs in control x100 [8].

The effect on soil-dwelling organisms was measured in a cubic meter area by extracting the soil, 30 days before and after the treatments. Natural enemies such as spiders, coccinellids, and chrysalids per plant were observed before and after in each treatment. The cost benefit ratio of the treatments has been calculated based on the formula given below.

BC Ratio =NR/CC; NR- Net Returns; CC- Cost of Cultivation

Data on percent mortality were calculated by Abbott's formula [9.]. Data on number of grubs were subjected to arcsine transformations and subjected to analysis of variance.

RESULTS AND DISCUSSION

Survey on sugarcane crop damage due to white grub and correlation with weather parameters

Survey on sugarcane crop damage due to white grub and correlation with weather parameters during 2017 was presented in Table 1 and Table 2.

Surveys on sugarcane crop drying in the East Godavari district of Andhra Pradesh from the onset of the monsoon rains in June and July of 2017-18 revealed that the white grub species *Holotrichia consanguinea* was responsible for sugarcane clump mortality.

Incidence was found in sugarcane fields that were planted or ratooned in the months of January to March 2017. Damage of white grubs was seen, and grubs were sampled by digging soil quadrats. The highest and most severe damage was detected in localized patches along field bunds and in the center of the field (Fig.1). Grubs devour sugarcane roots, reducing the amount of water and nutrients available to the plant. For any layman it is a chaotic task to distinguish and comprehend the basic difference amongst white grub damage symptom with the symptom of drought as visually both appear identical with initial yellowing and wilting of leaves resulting in drying and deterioration of mature stalks.

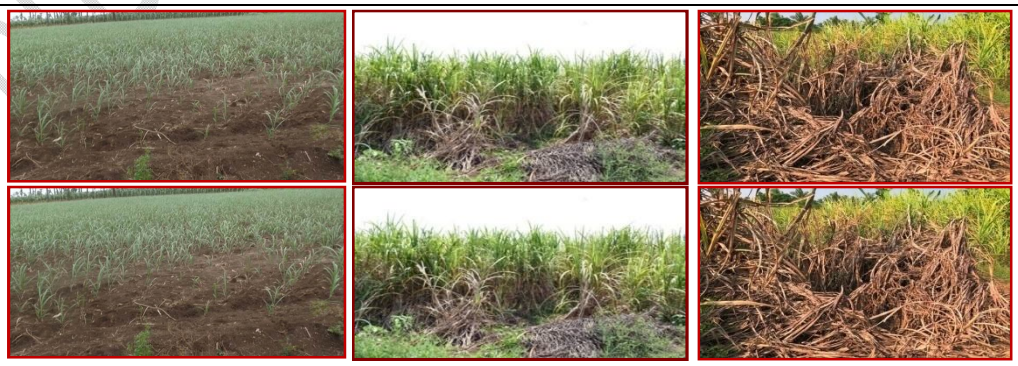

Fig1. Pictures showing White grub damage in sugarcane crop at different growth stages

Amongst four locations, observations recorded during second fortnight of July month, 2017 showed that sugarcane damage by white grubs was highest at Chebrolu village (Table 1). Sugarcane clumps due to white grubs recorded significantly higher damage in march ratoon crop (39.46%) as compared to February ratoon crop (13.61%); January plant crop (10.88%) and lowest in January seedlings (0.83%) in Chebrolu. Sugarcane clumps mutilation was significantly high in march ratoon crop (29.97%) in Rothulpudi village compared to February (14.1%) and January ratoon crops (4.25%).Whereas in P.Chamavaram and Boorampalem, damage was significantly high in marchratoon crop and on par with and February ratoon crop. Data on clump damage and age of the crop corroborated that grub damage was highest in march ratoon crop (4 months age); on the other hand, it was noticed lowest in January ratoon / plant crop (6 months age) and January seedling plant crop (7 months age).

Studies revealed that sugarcane clump damage and grubs count in chebrolu village, East Godavari district, Andhra Pradesh during 2017 indicates that increase in humidity (68% to75%) along with a decrease in temperature (3 to 5°C) during June, July and august months favor the emergence of beetles, its egg laying whichin turn resulted in severe clump damageby white grubs in July, August and September months i.e.,12.15 %, 14.33% and 19.2 % respectively (Table 2). Rainfall (12.36mm) and low relative humidity (<70 %) triggers adult emergence in Mayand increase in relative humidity(71% &-72 %) with high amounts of rain received during June(169.76 mm), July(227.3 mm) and August (112.25 mm) also instigated the emergence of beetles resulted in severe clump vandalization in the months of July to September months (12.15% to 19.2 %) .

The advent of beetle population commences primarily after the receipt of summer showers with a minimum level rainfall of 11mm sufficient for the adult emergence during the last week of May. As the name June beetles itself suggests the peak period of the beetle emergence in the second fortnight of June which is synonymous to the name of white grub and which continues till the fortnight of August [10].

The pre monsoon increase in atmospheric moisture is a characteristic of the monsoon arrival in India and is useful for forecasting and managing adult beetles [11,12,13]. Another instance of significant upsurge in grub population was correlated with increased relative humidity in case of teak[14].

Field efficacy studies

Field efficacy of entomopathogenic nematode and entomophagous against white grub are presented in Table- 3(2017-18), Table-4 (2018-19) and Table-5 (2019-20).

During the first-year field trial (2017-18), the per cent sugarcane clumps damaged by white grubs was lowest (7.44 %) in *H. indica* (NBAII H38) WP @ 12kg ha^{-1} (twice application) and superior to chlorantraniliprole 18.5 SC treatment @ 300 ml ha^{-1} (10.01 %) and *M. anisopliae* (NBAIR Ma4) 2.0% W.P. Formulation @ 2.5kgha^{-1} (twice application) treated plots (11.92 %) (Table 3). White grub population was significantly low (1.75 grubs/ 10 m row) in chlorantraniliprole 18.5 SC treatment compared to other treatments and untreated control recorded significantly high grub population of grubs/10m row (12.0 grubs/ 10 m row).

The second and third-year field trials showed the lowest per cent plant damage by white grubs was observed by the usage of *H. indica* (NBAII H38) WP @12 kg ha^{-1} (1.13% and 1.11 %) which was statistically at par with *M. anisopliae* (NBAIR Ma4) @ 2.5kgha^{-1}(1.27% and 1.31%) and untreated control recorded highest plant damage (6.0 % and 3.01%) during 2018-19 and 2019-20 (Table 4 and 5).

Mean number of grubs are high in untreated control and low in *H. indica* (3.22 and 3.01 grubs/10m row) and was on par with *M. anisopliae* (3.4 and 3.61grubs/10m row) and chlorantraniliprole 18.5 SC treatment(4.18 and 4.33 grubs/10m row)in second- and third-year trials.

Studies on field efficacy of *M. anisopliae* against white grub, *Leucopholis lepidophora* in sugarcane showed that *M. anisopliae*followed by entomopathogenic nematode application was studied in Maharashtra with minimal clump mortality [15].

Manisegaran *et al*. (2011)[16] reported that application of *M. anisopliae* at the rate of 4 $x10^9$ conidia $ha^{-1}$was effective against sugarcane white grub *Holotrichia serrata* (Blanch) with 92% reduction of grub population. Samson *et al*.[147] studied that soil application of *M. anisopliae* @ 3.3 x 10^{13}conidia ha^{-1} against gray black cane grub, *Dermolepida albohirtu* resulted in50-60 per cent reduction in grub infesting sugarcane in Australia. *M. anisopliae* and *B. bassiana* @ 5x 10^8 conidia ha^{-1}in combination with chlorpyriphos were applied @ 2lit. ha^{-1} which was found effective in reducing grub population [18]. In the first-year trial (2017-18), cane yield was significantly high (52.75 t/ha) with the usageof *H. indica* (NBAII H38) and was statistically at par with Chlorantraniliprole 18.5SC treatment (49.67 t/ha) as well *M. anisopliae* (NBAIR-

Ma4) 2.0% W.P. formulation @ 2.5kgha^{-1} treatment (48.89 t/ha). Untreated control recorded significantly exceptionally low cane yield of 13.65t/ha .

In the second and third year trials, the cane yield was significantly higher in *H.indica* (NBAII H38) treatment (65.44t/ha and 67.31 t/ha) which was statistically at par with Chlorantraniliprole soil application (64.26t/ha and 60.01 t/ha) and *M. anisopliae* (NBAIR-Ma4) (62.4t/ha and 63.21 t/ha) during 2018-19 and 2019-20. Untreated control recorded significantly low cane yields of 22.98t/ha and 59.10t /ha (2018-19 and 2019-20). The soil application of *M. anisopliae* @ 2 x 10^{12} conidia ha^{-1} was effective in the reduction of white grubs and higher yields [19].

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The cost benefit ratio was found highest in *H. indica* (NBAII H38) in three years study (1.05,1.46 and 1.49) which was followed by *M. anisopliae* (NBAIR Ma4) 2.0% W.P. formulation treatment (1.03, 1.41 and 1.39) and chlorantraniliprole treatment showed C:B ratio of 1.01 (2017-18), 1.34 (2018-19) and 1.31 (2019-20) (Table 3, 4 & 5). Percentage reduction in plant damage and grub population over control recorded high in *H. indica* (79.2% and 82.29%), *M. anisopliae* (67.74% and 76.04%) and chlorantraniliprole 18.5SC(72.91% and 85.42%) during 2017-18. Similarly, percentage reduction in plant damage and grub population over untreated control recorded high in *H. indica* (81.17% and 79.2%), *M. anisopliae* (78.83% and 78.04%) and Chlorantraniliprole 18.5SC (54.17% and 72.99%) during 2018-19. Likewise, similar trend was observedin 2019-2020 with Percentage reduction in plant damage and grub population over control recorded high in *H. indica* (79.18%), *M. anisopliae* (77.91%) and Chlorantraniliprole 18.5SC (52.01%) during 2019-20. Entomopathogenic nematode, *H. indica* and entomophagous, *M. anisopliae* proved effective in reducing white grub damage compared to insecticide, chlorantraniliprole 18.5SC treatment in all the consecutive years.

CONCLUSION

An estimation of damage is particularly useful in the early vegetative stage of sugarcane crop growth for adopting management practices in current season and also in subsequent growing seasons. It can be epilogued that as an alternative to insecticide chlorantraniliprole sorting to management practices using biocontrol agents such as Entomopathogenic nematode,

Heterorhabditis indica as well entomophagous , *Metarhizium anisopliae* are not only environmentally friendly but also yields in reducing white grub, *Holotrichia consanguinea* in higher cane yield compared to insecticide treatment viz., Chlorantraniliprole 18.5SC soil application. Based on cost-benefit ratio, the treatment with *M. anisopliae*, (NBAIR Ma-4) and *Holotrichia consanguinea*(NBAII H38) were found superior to Chlorantraniliprole18.5SC insecticidal application in the management of sugarcane white grubs in coastal Andhra Pradesh.Utilization of entomopathogenic nematodes and entomophagous for the management of white grubs is useful for the benefit of farming community. The higher fecundity rate of insects has compelled the farmers to sort for usage of chemical insecticides from ages old which over a period of time have led to devastating effects not only on the soil inhabiting fauna but also creating a considerable negative impact on non-target organisms as well creating further repercussions viz., environmental pollution and development of resistance in the insects (with) resulting in biotypes. Further, excessive dumping of pesticides at the nick of the moment in haste might end up in residue problems not only on the foliage but also on the soil inhabiting micro fauna. Hence this study involves in usage of native isolates of entomopathogenic nematodes (*Heterorhabditis indica* NBAII-H38), as well entomopathogenic fungus (*Metarhizium anisopliae* NBAIR Ma4 strain) extracted and evaluated for the efficient control of White grub menace. These are proved to be effective and at par with insecticidal application (Chlorantraniliprole) preventing the triggering of above mentioned consequences as a resource efficient and economical alternative .

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Table 1. Survey on sugarcane crop damage due to white grub in East Godavari district, Andhra Pradeshduring 2017-18

Plant Crop / Ratoon crop	Sugarcane clumps damage due to white grubs (%)				Number of white grubs / 10m row			
	Chebrolu*	Rothuloudi	P.Chamavaram	Boorampalem*	Chebrolu*	Rothuloudi	P.Chamavaram	Boorampalem*
January plant crop	10.88^{bc} (19.14)	4.95^{c} (0.594)	1.296^{d} (0.086)	2.634^{b} (0.556)	10.78^{bc} (19.08)	4.75^{c}	2.875^{c}	4.375^{bc} (12.0)
January seedling plant crop	0.83^{d} (3.16)	6.109^{c} (0.751)	6.354^{b} (0.758)	3.596^{b} (0.546)	1.38^{d} (4.07)	7.125^{c}	7.5^{c}	4.625^{c} (11.15)
January ratoon crop	5.84^{c} (13.83)	4.25^{c} (0.615)	3.706^{c} (0.554)	4.58^{b} (0.729)	7.25^{c} (15.51)	5.875^{c}	6.0^{c}	7.5^{b} (15.72)
February ratoon crop	13.61^{b} (21.19)	14.1^{b} (1.103)	17.759^{a} (1.234)	20.28^{a} (1.291)	12.88^{b} (20.77)	15.875^{b}	18.125^{b}	19.375^{a} (25.88)
March ratoon crop	39.46^{a} (19.14)	29.97^{a} (1.469)	26.41^{a} (1.386)	26.124^{a} (1.399)	24.5^{a} (29.36)	30.0^{a}	24.5^{a}	22.5^{a} (28.03)
CD (0.05)	6.60	0.172	0.155	0.214	4.51	5.29	5.47	4.67
CV%	33.65	18.57	18.82	23.1	24.79	40.56	45.27	39.05

Values in parenthesis are logarithmic transformed values.

*Values in parenthesis are arc sign transformed values

Table 2: Sugarcane white grub incidence and weatherin Chebrolu, East Godavari district, Andhra Pradesh during 2017-18

Observation period Monthly	Sugarcane clumps damage due to white grubs (%)	Number of white grubs / 10m row	Rainfall (mm)	Rainy days	Temp^{0}C Max	Temp^{0}C Min	RH %	Sunshine hours	Sunny days
April	0.0	0.0	7.92	5	36	27	72	361.5	17
May	0.0	0.0	12.36	6	37	29	68	381.5	17
June	0.0	0.0	169.76	26	34	28	71	282.5	2
July	12.15	9.96	227.3	24	33	28	72	253.0	6
August	14.33	12.47	112.25	24	32	27	75	301.5	5
September	19.2	15.18	149.4	24	32	27	78	313.0	4
October	26.14	20.65	106.39	7	31	28	75	287.0	13
November	29.06	23.0	34.28	13	29	26	71	208.5	15
December	36.95	26.22	1.94	1	28	24	67	228.5	27

* Temp: Temperature; RH : Relative humidity; FN: Forenoon; AN: Afternoon; stdw: standard week

Table 3. Efficacy of entomopathogenic nematodes and entomophagous for the management of white grub in sugarcane during 2017-18

Treatment	Sugarcane clumps damage due to white grubs (%)*	Percentage reduction in white grub damage over control	Number of white grubs / 10m row	Reduction in grub population over control (%)	Cane Yield t/ha	Yield increase over control (%)	Cost Benefit ratio
T1: *Heterorhabditis indica* WP NBAII H38 @12 kg/ha in 150 kg sand per ha	7.44	79.86	2.125	82.29	52.75	74.12	1.05
T2: *Metarhizium anisopliae*NBAIR Ma 4 @ 5 kg/ ha in 250 kg FYM per ha	11.92	67.74	2.875	76.04	48.89	72.08	1.03
T3: Chlorantraniliprole 18.5SC @ 0.3 ml / lit	10.01	72.91	1.75	85.42	49.67	72.52	1.01
T4 : Untreated control	36.95		12.0		13.65		
CD (0.05)	1.05		0.255		5.20		
CV%	26.05		18.05		12.05		

DAT : days after treatment ; MAT : months after treatment

Table 4. Efficacy of entomopathogenic nematodes and entomophagous for the management of white grub in sugarcane during 2018-19

Treatment	Sugarcane clumps damage due to white grubs (%)	Percentage reduction in white grub damage over control	Number of white grubs / 10m row	Reduction in grub population over control (%)	Cane Yield t/ha	Yield increase over control (%)	Cost Benefit ratio
T1: *Heterorhabditis indica* WP NBAII H38 @12 kg/ha in 150 kg sand per ha	1.13	81.17	3.22	79.20	66.50	65.44	1.46
T2: *Metarhizium anisopliae*NBAIR Ma 4 @ 5 kg/ ha in 250 kg FYM per ha	1.27	78.83	3.40	78.04	64.29	64.26	1.41
T3: Chlorantraniliprole 18.5SC @ 0.3 ml / lit	2.75	54.17	4.18	72.99	61.11	62.40	1.34
T4 : Untreated control	36.0		15.47		22.98		
CD (0.05)	0.43		1.41		5.12		
CV%	34.01		31.22				

DAT : days after treatment ; MAT : months after treatment

Table 5. Efficacy of entomopathogenic nematodes and entomophagous for the management of white grub in sugarcane during 2019-2020

Treatment	Sugarcane clumps damage due to white grubs (%)	Percentage reduction in white grub damage over control	Number of white grubs / 10m row	Reduction in grub population over control (%)	Cane Yield t/ha	Yield increase over control (%)	Cost Benefit ratio
T1: *Heterorhabditis indica* WP NBAII H38 @12 kg/ha in 150 kg sand per ha	1.11	79.18	3.01	76.34	68.32	67.31	1.49
T2: *Metarhizium anisopliae*NBAIR Ma 4 @ 5 kg/ ha in 250 kg FYM per ha	1.31	77.91	3.61	73.45	67.99	63.21	1.39
T3: Chlorantraniliprole 18.5SC @ 0.3 ml / lit	3.01	52.01	4.33	71.87	59.10	60.01	1.31
T4 : Untreated control	35.85		14.38		21.74		
CD (0.05)	0.41		1.38		4.68		
CV%	33.02		29.69				

DAT : days after treatment ; MAT : months after treatment