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BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE *Meloidogyne javanica* ON SUNFLOWER PLANTS BY *Trichoderma album* AND *Bacillus megaterium*

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ABSTRACT

Two Egyptian bioagents i.e. Biozeid ™ (*Trichoderma album* in 10 x 10^6 spores / g.) and Bioarc ™ (*Bacillus megaterium* in 25 x 10^6 cfu / g.) were evaluated as bio-nematicides at the rate of 1.2, 2.0, 5.0,10.0 15.0, 20.0, 25.0 and 30.0 g. /l. against *Meloidogyne javanica* (j2) under laboratory conditions (25 ± 2 ºC) . In additions, three rates of both bioagents 20.0, 25.0 and 30.0 g. /l. and a mixture of them (10 g *T. album* + 10 g. *B.megaterium* /l.) in comparison with the nematicides, Oxamyl (Vaydate 24 %) were then tested against *M. javanica* infecting sunflower cv. Giza 35 as well as plant growth response under greenhouse conditions(30 ± 5 ºC) . *In Vitro*, all tested rates of both bioagents have pronounced activity against *M. javanica* (j2), their bio-efficacy were positively increased as exposure time and rates increased , since *T. album* or *B. megaterium* gave 85.9 % or 80.7 % mortality of *M. javanica* (j2) at rate 30.0 g. /l after 96 h. Under greenhouse conditions, all tasted treatments showed significant reduction in rates of nematode reproduction as well as increased in the reduction percentage of nematode population whereas, Oxamyl achieved the lowest value of rate of nematode reproduction (0.07) and the highest value of nematode reduction percentage (95.0 %). Non significant differences were recorded between the two highest rates (25.0, 30.0 g / l) of both bioagents, *T. album* and *B. megaterium* or their mixture or Oxamyl in this respect. Moreover, these two rates and Oxamyl treatments, also played an important role in the improvement of plant growth parameters as compared to nematode alone. It was concluded that both bioagents tested at the highest rate of 30 g/l. obviously improved plant growth in sunflower and reduced nematode reproduction and density.

Keywords: Biozeid *, Trichoderma album*, Bioarc, *Bacillus megaterium* , *Meloidogyne javanica,* bioagents and sunflower plant.

INTRODUCTION

Plant parasitic nematodes cause great economic losses to agricultural crops world wide (Sasser and Freckman,1987) and are considered an economically important group of soil borne pathogens that may be controlled by cultural practices, chemical nematicides and the use of resistant cultivars. However, nematicides do not long-term suppression of nematodes, and environmental and human health concerns are resulting in increasing restrictions in their use (Perez *et. al.,* 2003). Therefore, there is still need for alternatives, environmentally friendly or compounds for effective nematode control (Noling & Becker, 1994). So, certain safe procedures for nematode control have been developed based on bio-control agents and organic amendments, Several attempts have been made to use *Trichoderma* spp. or *Bacillus* spp. to control plant parasitic nematodes by reducing egg production of root-knot nematodes which infected roots of several hosts. Besides, the penetration of root–knot nematodes were suppressed by several

isolation of *T.lengnorum , T. harzianum* or *B. cereus,* (Windham *et. al.,*1989; Spiegel & Chet ,1998; Nagesh *et. al.,*2005 and Khyami-Horani & Al-Banna, 2006).

Trichoderma album and *Bacillus megaterium* have been used as bio-control agents against soil borne, foliar and post harvest phytopathogenic fungal pathogens (Abou-zeid & zaid, 2006). Bin *et. al.*(2005) reported that the rhizobacteria identified as *Brevibacillus brevis* and *Bacillus subtilis* do not only inhibited the radial growth of the root-infecting fungi *Rhizoctonia solani* , *Pythium aphanidermatum* and *Fusarium oxysporum* , F. *cucumerinum in Vitro,* but also exhibited strong nematicidal activity by killing in different levels the second stages juveniles of *M. javanica* in greenhouse.

The present work was carried out to evaluate two Egyptian bio-agents fungicides namely;

Biozeid W.P (*Trichoderma album* in 10 x 10^6 spores / g.) and

Bioarc W.P (*Bacillus megaterium* in 25 x 10^6 cfu / g.) at eight rates to testify their efficacy on *M. javanica* (j 2) *in Vitro* as well as the impact of both bio-agents at two either alone or a mixture of them in comparison with Oxamyl on *M. javanica* infecting sunflower plants under greenhouse conditions.

MATERIALS AND METHODS

Biological Control Agents

Two new biocontrol agents produced by unit the of Identification of Microorganisms and Biological control, plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt were evaluated against the root – knot nematode, *Meloidogyne javanica In vitro* and under greenhouse conditions. The bio-agents were,

Biozeid ™ (*Trichoderma album* ,10 x 10^6 spores / g.) and

Bioarc ™ (*Bacilluc megaterium*, 25 x 10^6 colony forming unit (cfu) / g.)

A - *In Vitro* Experiment

Eight rates of both *T. album* and *B.megaterium* were prepared and separately tested against each of one hundred freshly hatched juveniles of *M . javanica* in Petri dishes (5 cm. diameter). These rates were (1.2, 2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 g. /l.). The examination was followed according to schedule time of exposure (24, 48, 72 and 96 hours). Petri dishes containing distilled water served as control and each treatment were replicated three times, and the incubation at 25 ± 2 º C was done. Juveniles of *M. javanica* that exhibited no perceptible movement under stereomicroscope were considered as dead, counted and the corrected mortality (immobility stages) percentage was calculated according to Abbott formula, (Abbott, 1925).

B – In Greenhouse Experiment

This work was under taken in greenhouse of Nematology Dept., Plant Pathology Res. Inst. , Agric. Res. centre, Giza, Egypt.

Four seeds of sunflowers, *Helianthus annuus*, Cv. Giza 35 were planted in each pot measured 20 cm. diam. Two week after germination, seedlings were thinned to one healthy plant per pot, then the pots were divided into treatments according to the following bio-agents rates, 20 , 25 and 30 g./l. of both *T. album* , *B. megaterium* and a mixture of them in rate of 10 g *T. album* + 10 g. *B.megaterium* /l. volume of 30 cm^3 of each bio-agents were added to each of four pots (treatment). After 10 days of application with bio-agents, each pot was inoculated with 3000 fresh newly hatched juveniles of *M . javanica* .

The nematicide, Oxamyl (Vaydate 24 %) at the rate of 0.05 % was used as standered indicator beside untreated four pots that served as control. The experiments were arranged in a complete in randomized design under greenhouse conditions (30 ± 5). After two months, plants were harvested and the obtained data on plant growth parameters and nematode population in both soil and root were recorded. Number of second stage juveniles of *M . javanica* per 250 g. of soil were estimated (Goodey, 1957). Number of developmental stage, young females and egg-laying females per the whole root system were also estimated, and the rate of nematode reproduction were then calculated and recorded . Root gall index was determined by using the scale of (Taylor and Sasser, 1978) and the reduction percentages on nematode population was determinated as well. The increasing percentages in plant growth parameters for the fresh weight of the whole plant and dry weight of the shoot were also calculated. Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez , 1984) and means were compared by Duncan´ s multiple – range test.

RESULTS AND DISCUSSION

A- The bio- effect of *T. album* and *B. megaterium* against *M . javanica* in *vitro.*

The bio-agents , *T. album* and *B. megaterium* presented remarkable nematotoxic activity against second stages juveniles *M . javanica*, (Figure 1&2) .

Results illustrated in Fig. (1&2) showed that the mortality (immobility) increased with the increase of both rates and exposure periods. More than 50 % mortality was recorded with *T. album* at rate of 20 g. / l. (50.8 %) , while the other bioagent (*B. megaterium*) attained
(59.2 %) mortality at rate of 25 g . / l. through 24 h. of exposure period. This mortality percentage was resulted from *T. album* 57.2 %) or *B. megaterium* (56.4 %) at lower rates (15 g. / l.) after 96 h. exposure period. This intermediate periods of exposure periods of exposure (48 & 72 h.) attained similar mortality level with application rate of 20 and 15 g. / l. for *T. album* respectively. However, this influence was recorded in the case of *B. megaterium* at the rate of 20 g. / l. for both two exposure periods, respectively. It also appeared from Fig. (1&2) that the two bio-agents reached their highest activity against *M . javanica* juveniles at the rate of 30 g. / l. period. These percentages of (j 2) mortality were 85.9 % and 80.7 % for *T. album* and *B. megaterium* respectively.

B-The bio- effect of *T. album* and *B. megaterium* against *M . javanica* Infecting sunflower plant under greenhouse conditions (30 ± 5).

Data in Table (1**)** represent the effect of bio-agents (fungus or bacteria) and oxamyl on nematode population infested sunflower plants. For root gall index, oxamyl and *T. album* at 30 g./ l. recorded the best results with no significant differences between them . However, *T. album* 25 g. / l. and *B. megaterium* at 30 g./ l. and their mixture (10 g *T. album* + 10 g. *B.megaterium*) positioned the second level for this pattern (Table 1). In third sit, of root gall index *T. album* at 20 g./l. or *B. megaterium* at 20 or 25 g./l. were tested. It is also evident that all treatments stage juveniles nematodes *M. javanica* in soil and root of sunflower plants with a rate of nematode build-up ranged from 0.07 of oxamyl to 0.33 for *B. megaterium* against 1.41 for untreated plants (nematode alone) . However, there are significant differences between bioagents application. The best reduction in nematode population was obtained with oxamyl (95.0 %) with no significant differences with the application of *T. album* and *B. megaterium* at rates of 25 and 30 g./ l. or their mixture (10 g *T. album* + 10 g. *B.megaterium*). The effective percentages for *T. album* ranged from 78.0 to 90.1 % and for *B. megaterium* from 83.7 to 89.4 % at the proceeding rates. The mixture of two bio-agent attained 89.4 as well. However, the bio-agents *T. album* and *B.megaterium* at 20 g./l. gave the least values of nematode population where the reduction percentages ranged between 76.6 % for *B.megaterium* and 78.0% for *T. album* .

Data illustrated in Fig. (3 & 4) represent the bio-agents effect on growth parameters of infected sunflower plants with *M. javanica* juveniles under greenhouse conditions. It is clear from the illustration that, the most treatments were more benefit in improving the growth of sunflower plant to a certain extent, exceeded that of nematode alone. Concerning fresh weight of the whole sunflower plant, a high percentage of increase was recorded with oxamyl, *T. album* at rate 30 g./l. and *B. megaterium* at 30 g./l. and mixture of them (10 g *T. album* + 10 g. *B.megaterium*). However, the rate of 20 g./l. for both *T. album* and *B.megaterium* had the lowest increasing percentage in this respect. The same trend was obtained with the increasing percentages in shoot dry weight of sunflower plant as compared with nematode alone.

Form the previous results it could be concluded that the two tested (*T. album* and *B.megaterium*) had remarkable nematotoxic activity against *M. javanica* in *vitro* where the immobility percentages increased by increasing the period of exposure (hours) and rate of application. The present findings were supported by Chet and Inbar ,1994 & Dhawan *et.al.*, 2004. Regarding the experiment under greenhouse conditions, the two bio-agents (*T. album* and *B.megaterium*) were varied in their effectiveness as bio-nematicides when used at different rates. Higher depression in nematode percentages reproduction of *M. javanica* was achieved by *T. album* , *B.megaterium* at rate 30 g./l. and their mixture. These results were in agreement with those obtained by Cherif and Benhamou , 1990 & Tronsmo *et.al.*, 1993. They recorded that *Trichoderma* spp. considered a good source of various toxin and may be produce lytic enzymes. So it may be plays an important role in dissolving the nematode egg layers, consequently abortion the hatching of

the eggs. Spiegel, *et. al.*, 2005, were intensively studied the mode of action of *Trichoderma* spp. as biocontrol of root-knot nematodes. They concluded that the direct fungal parasitism of the spores to nematode and diffuse of their lytic enzymes which stimulated with special genes. These enzyme genes were turned on during the interaction between the fungus and the nematodes. So, a significant enhancement of fungal parasitism on the nematode was done. Similar mode of action of *Bacillus* spp. was noticed by Niu-qiu Hong, *et .al.*, 2006, when extracted the crude extra-cellular protein from culture of bacteria. This extraction killed about 80 % of the tested nematode within 24 h. The effectiveness attributed to presence of extra-cellular proteases. They also found that the purified protease can hydrolyze several native proteinaceous substrates, including collagen and nematode cuticle. Therefore, a significant reduction in root gall index which observed on root of plants infected with root- knot nematodes that caused by *B. megaterium ,T. harzianum* and *T. viride* recorded by Begum and Kumar , 2005; Pandey , 2005 & Padgham and Sikora , 2006. Beside, this result the percentages increase in both of fresh weight of the whole plant and dry shoot weight which obtained by using bio-nematicide agents supported by Haseeb *et. al.*, 2005 & Khan *et al.*, 2005.

Table (1): Effect of Biozeid (*T. album*) and Bioarc (*B. megaterium*) rates on *M. javanica* population infested some flower plants under greenhouse conditions

Treatments	Rates g./l.	Root gall index	Nematode population				Final population	Rate of reproduction (Pf/Pi)	Reduction %
			Juveniles in soil/ pot	In root					
				Develo-pmental stages	Young females	Egg-laying females			
Biozeid	20	3.0 b	209.2 b	35.3 b	34.9 b	32.6 bc	312 b	0.31	78.0
	25	2.0 c	140.2 c	18.2 cd	16.4 cd	15.9 c	190.7 bc	0.19	86.5
	30	1.0 d	107.8 d	11.2 de	10.2 de	13.4 cd	142.6 bc	0.14	90.1
Bioarc	20	3.0 b	227.6 b	37.2 b	35.9 b	34.2 b	334.9 b	0.33	76.6
	25	3.0 b	159.6 c	25.3 c	21.0 c	26.2 b	232.1 bc	0.23	83.7
	30	2.0 c	115.3 d	13.0 de	12.3 de	14.2 c	154.8 bc	0.15	89.4
Biozeid + Bioarc	10+10	2.0 c	112.3 d	12.2 de	14.3 de	15.7 c	154.5 bc	0.15	89.4
Oxamyl(Nematicid)	Rec.	1.0 d	54.0 e	6.4 e	8.2 e	5.3 d	73.9 c	0.07	95.0
Control+Nematode	0	5.0 a	1054.2 a	235.8 a	69.2 a	47.2 a	1406.4 a	1.41	

*** Each value presented the mean of four replicates**

*** Pi = 3000**

Means in each column followed by the same letter did not differ at < 0.05 according to Duncan's multiple test.

In conclusion, the bio-effectiveness of treatments with *T. album* and *B.megaterium* at higher rate (30 g. /l.) resulted an improvement in sunflower growth and reduced the reproduction rate for *M. javanica* infected sunflower plants, indicated a promise for test (Biozeid) *T. album* and (Bioarc) *B.megaterium* at rates above 30 g. /l . per secure meter in open field as a bio-nematicides.

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المقاومة الحيوية لنيماتودا تعقد الجذور مليودوجين جافينكا المتطفلة على نباتات دوار الشمس باستخدام فطر تريكوديرما البيوم و بكتريا باسيلس ميجاتيريم .

ايمان السيد حماد , على محمد على زيد
معهد بحوث أمراض النباتات- مركز البحوث الزراعية- وزارة الزراعة – الجيزة – مصر.

هذا البحث هو محاولة لإلقاء مزيداً من الضوء حول مكافحة نيماتودا تعقد الجذور بطريق حيوية . تم تقييم فعالية اثنين من المركبات الحيوية المصرية و هما مركبى بيوزيد وبيوارك لمكافحة نيماتودا تعقد الجذور فى نبات دوار الشمس. يحتوىالمركب الحيوى بيوزيد على مادة فعالة عبارة عن فطر ترايكودرما ألبيوم يحتوى الجرام منه على ١٠ × ١٠^{٦} جرثومة ومركب بيوارك يحتوى مادة فعالة عبارة عن بكتريا باسيليس ميجاتيريم يحتوى الجرام منه على ٢٥ × ١٠^{٦} وحدة مكونة للمستعمرة.

أظهرت النتائج المعملية للتركيزات المختبرة (١,٢, ٢,٥ ,٥,٠ , ١٠,٠ , ١٥,٠ , ٢٠,٠ , ٢٥,٠ و ٣٠,٠ جرام /لتر) على معدلات الموت ليرقات نيماتودا تعقد الجذور و جود علاقة طردية بين فترة التعريض للمركبات بالتركيزات المستخدمة و زيادة معدلات الموت لليرقات , حيث اعطى كل من باسيليس ميجاتيريم و تريكوديرما ألبيوم ٨٠,٧ – ٨٥,٩ ٪ نسبة موت ليرقات نيماتودا تعقد الجذور عند معدل ٣٠ جرام / لتر لفترة تعريض ٩٦ ساعة .

كما تم استخدام ثلاثة تركيزات ٢٠ , ٢٥ و ٣٠ جرام /لتر من كل من المركبين المختبرين مع خليط لهما بنسبة ١٠ جرام تريكوديرما ألبيوم + ١٠ جرام باسيليس ميجاتيريم مقارنة مع المبيد النيماتودى الأوكساميل ٢٤ ٪ سائل بالتركيز الموصى به و ذلك على نباتات دوار الشمس صنف جيزة ٣٥ التى تم عمل عدوى صناعية بمعدل ٣٠٠٠ يرقة من نيماتودا تعقد الجذور تحت ظروف الصوبة الزجاجية (٣٠ ± ٥ ^{o} م) و بعد مرور شهرين من بدء التجربة أعطت جميع المعاملات المختبرة زيادة معنوية فى خفض اعداد النيماتودا فى كل من التربة و الجذور لنباتات دوار الشمس مما يترتب عليه خفض معدل التكاثر للنيماتودا, و بالأخص عند معدل ٣٠ جرام /لتر لكل من المركبين المستخدمين مع الخلط بينهما عند مقارنتهما بالتركيز الموصى به لمبيد الأوكساميل حيث كان نسبة معدل الخفض فى معدل التكاثر لنيماتودا تعقد الجذورتتراوح بين ٨٩,٤ ٪ لكل من باسيليس ميجاتيريم و الخليط بينهما (١٠ جرام تريكوديرما ألبيوم + ١٠ جرام باسيليس ميجاتيريم) و ٩٥ ٪ لمبيد الأوكساميل . أيضا حدث خفض معنوى فى معدل تكوين أكياس البيض لنيماتودا تعقد الجذور على جذور نباتات دوار الشمس و ذلك عند مقارنتها بالكنترول حيث كان لكل من المركبين المستخدمين دورا رئيسا فى تحسين صفات النمو المختبرة للنباتات لكل من المجموع الخضرى و المجموع الجذرى لدوار الشمس عند مقارنة تأثيرهما بتأثير مبيد الأوكساميل و معاملة الكنترول .

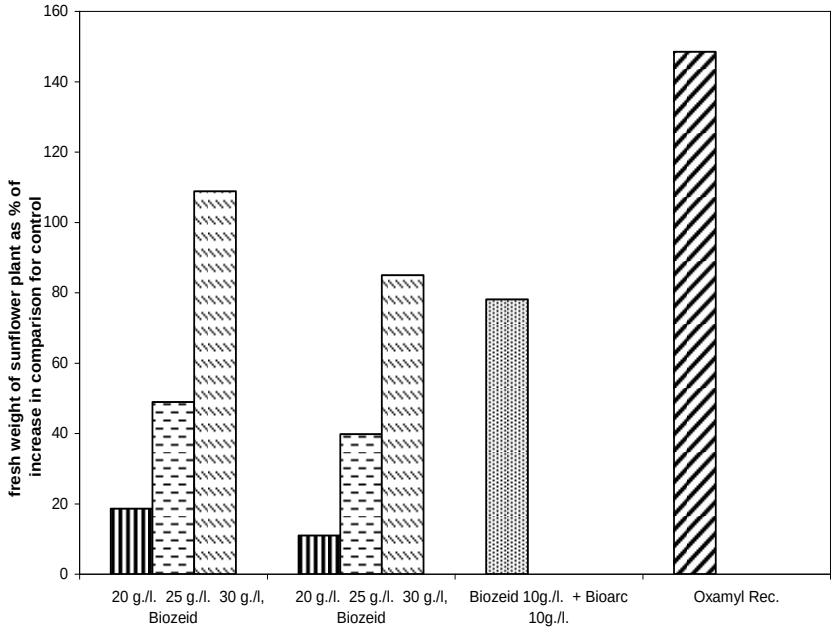

Fig.3. Effective of percentages of Biozeid (*T. album*) , Bioarc (*B. megaterium*) , mixture their and Oxamyl on fresh weight of sun flower plant

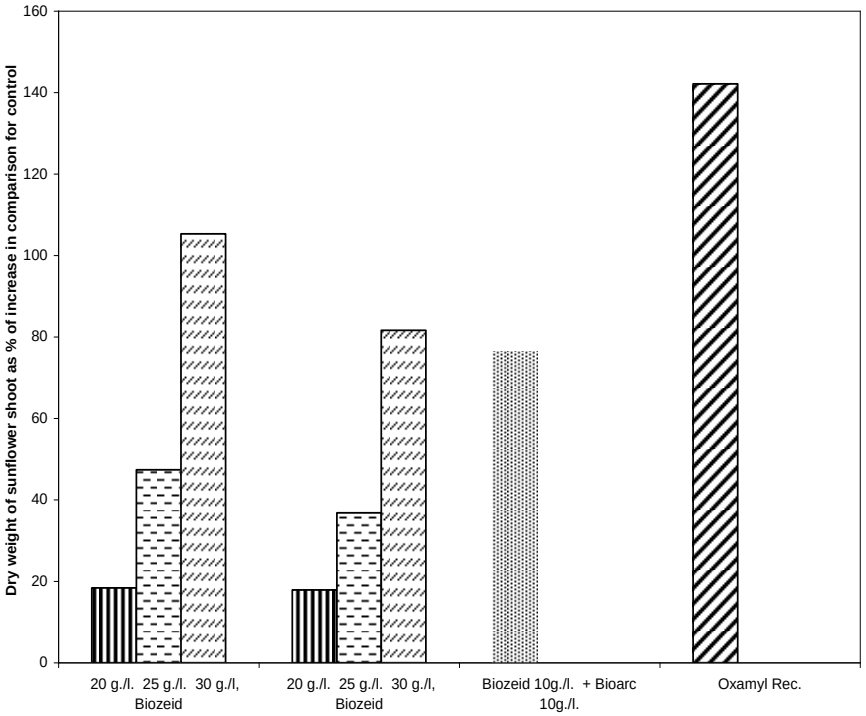

Fig. 4 . Effective of percentages of Biozeid (*T. album*) , Bioarc (*B. megaterium*) , Mixture their and Oxamyl on sunflower shoot dry weight

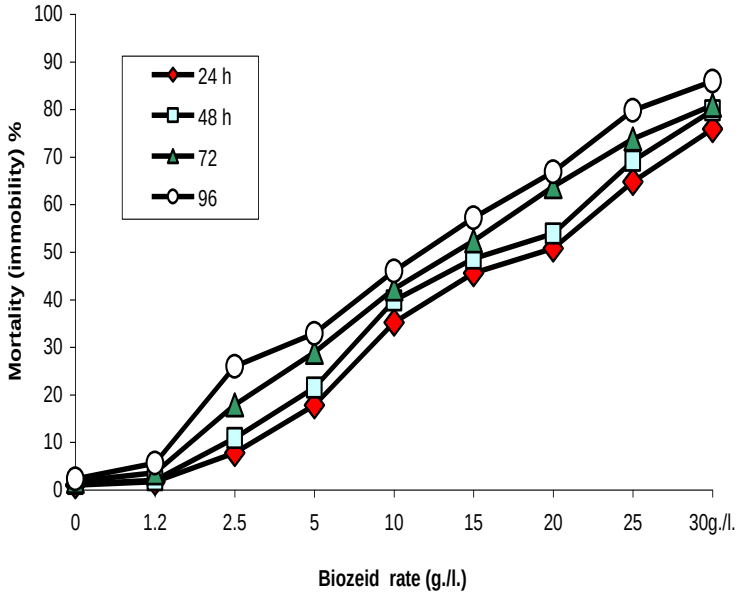

Fig.1. Effect of Biozeid (*T. album*) rates on mortality (immobility) percentage of *M. javanica* juveniles under laboratory conditions.

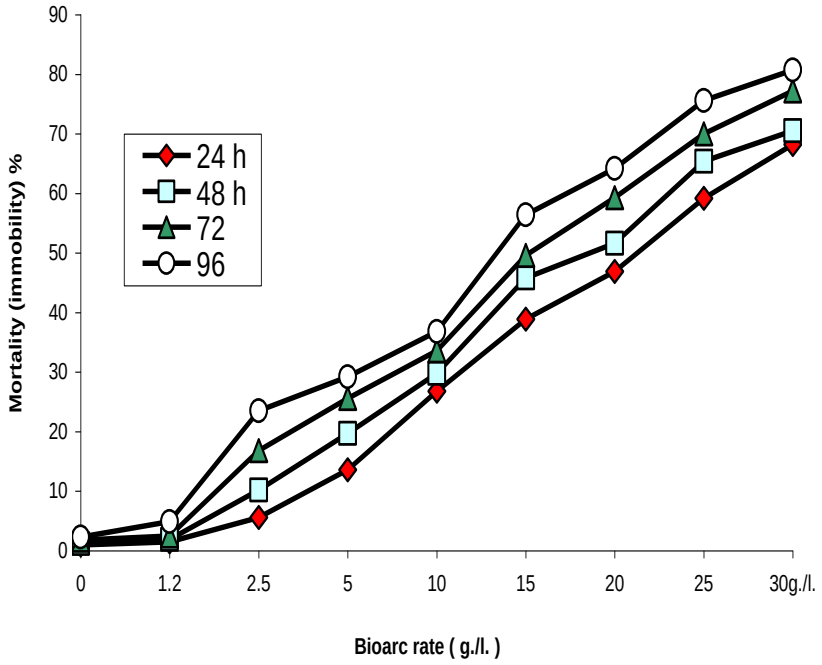

Fig. 2. Effect of Bioarc (*B. megaterium*) rates on mortality(immobility) percentage of *M. javanica* under Laboratory conditions