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COMPARATIVE EFFICACY OF DIFFERENT POPULATIONS OF *STEINERNEMA CARPOCAPSAE* AGAINST *SPODOPTERA LITURA* ON TOMATO

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ABSTRACT

Screen house studies was carried out to evaluate the efficacy of different populations of *S. carpocapsae* which was multiplied on different artificial media against 4th instar larvae of tobacco caterpillar (*Spodoptera litura* Fab.) on tomato. Mass multiplication of *S. carpocapsae* on different artificial media *i.e.*, Nutrient broth, Wheat flour, Maize flour, Lipid and Modified Wout's media. Populations of *S. carpocapsae* recovered from different artificial media with inoculum levels *i.e.*, 5000, 10000, 15000 and 20000 IJs/Plant. Maximum (80) per cent mortality of *S. litura* was observed after 168 hrs at an inoculum level of 20,000 IJs/plant recovered from Nutrient broth media followed by 77.5 and 75 per cent mortality at 20,000 IJs obtained from Modified Wout's and Lipid media respectively. However, minimum 40 per cent mortality recorded at 5,000 IJs produced on Wheat flour media.IJs of *S. carpocapsae* multiplied on different media, the population obtained from animal protein based media (Nutrient broth) had highest infectivity against *S. litura* under cage house condition than plant protein based media (Wheat flour).The differences in the rate of nematode infection and insect mortality under various experimental conditions are attributed to the difference in the behavior, virulence, rate of penetration and host searching abilities of nematodes.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is second most important remunerable Solanaceous vegetable crops after potato either for local consumption and exportation (Sahu *et al.,* 2013). It is protective supplementary food and considered as important commercial and dietary vegetable crop. As it is short duration crop and gives high yield, it is important from economic point of view and hence area under its cultivation is increasing day by day (Pedapati *et al.,* 2013). Its consumption is highly correlated with reduced risk of cancer and also low incidence of some cardiac diseases, due to some important constituents present in the fruit (Franceschi *et al.,* 1994). The most important of these are carotenoids, particularly lycopene and â- carotene which are accumulated in plasma and tissues in relation to the intake of tomatoes (Oshima *et al.,* 1996). However, crop having such economic value is unfortunately adversely affected by various diseases caused by fungi, bacteria, viruses, insects and nematodes. The key insect pests of tomato include Aphid (*Aphis gossypii* Glover), Jassid (*Amrasca devastans* Ishida), White fly (*Bemisia tabaci* Genn.), Leaf miner (*Liriomyza trifolii* Burgess), Thrips (*Scirtothrips dorsolis* Hood) and Fruit borer (*Helicoverpa armigera* Hub.) Chaudhuri and Senapati (2001), Reddy and Kumar, (2004).

Spodoptera *litura* (F.) (Lepidoptera: Noctuidae), commonly known as tobacco caterpillar in India is a major polyphagous pest attacks variety of economically important crops such as cotton, groundnut, rice, tomato, tobacco, citrus, cocoa, potato, rubber, castor, millets, sorghum, maize and many other vegetables (Hill, 1993). Larvae cause significant damage to both the foliage and developing fruits of field grown tomatoes (Muthukumaran and Selvanarayanan, 2008). Most studies on the economic impact of *S. litura* have been conducted in India, where it is a serious pest of a variety of field crops. It has caused 12 to 23% loss to tomatoes in the monsoon season, and 9 to 24% loss in the winter (Patnaik, 1998). The pest occurs in India, Pakistan, Bangladesh, Sri Lanka, S. E. Asia, China, Korea, Japan, Philippines, Indonesia, Australia, Pacific Islands, Hawaii and Fiji (Hill, 1993).

In various species of lepidopteran pest management, increasing failures of chemical pesticides and the problems posed by their indiscriminate use in the field have created a momentum to develop environment friendly methods of pest control. Among the alternatives that are currently available is the use of entomopathogenic nematodes (EPN) (Rhabditida: Steinernematidae and Heterorhabditidae) remain the most promising, considering the fact that they can be used in a manner similar to the familiar chemical pesticides. Heavy infestations and associated economic losses warrant the development of sustainable and environmentally safe strategies, such as the use of biocontrol agents like entomopathogenic nematodes (EPN) against lepidopteran pest.

The EPN species of *Steinernema* Travassos and *Heterorhabditis* Poinar are obligate pathogens of insects (Poinar, 1979). These groups of insect pathogens have been studied extensively in different biocontrol programmes (Ehlers, 2001). The free-living infective juveniles (IJs) have to locate a potential host, penetrate through its cuticle or natural openings and establish in the host's body cavity (Koppenhofer *et*

al., 2007). In the insect haemocoel, the nematode releases its symbiotic bacteria from its intestine. The bacteria proliferate, producing toxins and metabolites that kill the insect host and prevent invasion by secondary organisms. The nematodes feed on the lysed tissues of the host and bacterial colonies (Griffin *et al.,* 2005).

Safety and environmental issues surrounding the use of chemical insecticides has led to an emphasis on developing alternative control measures such as entomopathogens and their products. EPNs have been effectively used as biological insecticides in pest management programs (Grewal *et al.,* 2005) as they are considered nontoxic to humans, relatively specific to their target pest(s) and can be applied with standard pesticide equipment (Shapiro-Ilan *et al.,* 2006).

EPNs can be used as inundative or inoculative biological control agents or the proteinaceous toxins produced by their symbionts can be transferred to or/and expressed in crop plants or other microorganisms (Shapiro-Ilan *et al.,* 2012b). The IJ of EPNs are compatible with other biological and chemical pesticides, fertilizers, and soil amendments (Krishnayya and Grewal, 2002). The symbiotic association of bacteria with nematode makes it challenging for the insect to develop resistance.

The present investigation was designed with the objective to determine the comparative efficacy of entomopathogenic nematode (*Steinernema carpocapsae*) which multiplied on various media to find the infectivity of *S. carpocapsae* against *Spodoptera litura* infecting tomato under cage house conditions.

MATERIALS AND METHODS

The efficacy of entomopathogenic nematodes, *Steinernema carpocapsae* Steiner was tested against fourth instar larvae of *Spodoptera litura* (Fab.) at the four nematode inoculum levels i.e., 5000, 10000, 15000, 20000 infective juveniles/Plant and control (water without IJs). The bio-efficacy of *S. carpocapsae* recovered from different artificial media was studied against *S. litura* on tomato through spray in pots at different inoculum levels.

Nematode cultures

Steinernema carpocapsae was reared on the fifth instar larvae of greater wax moth*, Galleria mellonella* (L) under laboratory conditions (Wood ring and Kaya, 1988). This population was further multiplied on different artificial diet (Singh, 1994) i.e., Nutrient broth, Wheat flour, Maize flour, Lipid and Modified Wout's media under laboratory conditions at $25 \pm 1°C$ in BOD. A stock suspension of infective juveniles was made in sterile distilled water and used against *S. litura*.

Insects

The original population of *S. litura*, collected from cotton field in MPUAT, Udaipur. *Spodoptera litura* was reared on the leaves of castor, *Ricinus communis* (L.) in the laboratory under hygienic conditions at room temperature ($27 \pm 1°C$) and fourth instar larvae were used for treatments. Prior to the experiment, the insect larvae were washed three times with distilled water to remove soil and organic particles.

Sterilization of soil

Soil was steam sterilized in an autoclave. Two parts field soil, one-part sand and one part well ground FYM were thoroughly mixed. The mixture was then passed through an ordinary coarse sieve (16 mesh) in order to remove stones and pebbles. This mixture was then steam sterilized in an autoclave for two hours at 15 PSi-121ºC. Sterilized soil was kept in the cage house for at least 15 days for maturation and then used experimental purpose.

Disinfection, filling of pots and Planting

Earthen pots were washed, cleaned and disinfected before use by rinsing them through four per cent formalin solution. The formalin was allowed to evaporate before their use for experimentation. In all the experiments earthen clay pots of 30 cm diameter were filled with 5 kg soil. Tomato (var. Selection-120) seedlings (20 days old) were transplanted in each pot; treatments were replicated four times and watered regularly. Care was taken right from sowing or transplanting till the entire duration of experimentation.

The IJs were sprayed on infested crop according to the treatments using Knap sack sprayer. Other nutritional and plant protection practices such as weed management and disease management were undertaken following recommended package of practices. The per cent mortality of insect larvae was recorded on every 12 hrs up to 168 hrs after application. The dead insect larvae were collected and kept for the release of IJs on White trap (White, 1927) to check the pathogenicity. All the treatments were replicated four times.

RESULTS AND DISCUSSION

In pot conditions, no mortality of *S. litura* was found on 12 and 24 hrs after application. However, on 36 hrs maximum (7.50 %) per cent mortality of *S. litura* larvae at 20000 IJs/pot population multiplied on Nutrient broth media and Modified Wout's media and lowest mortality (5%) was found in at same dose population multiplied on all three media i.e., Lipid, Maize flour and Wheat flour media respectively. After 168 hrs maximum (80.0) per cent mortality was observed at 20000 IJs of *S. carpocapsae* recovered from Nutrient broth media followed by 77.50 and 75.0 per cent mortality was recorded at 20,000 IJs recovered from modified Wout's and lipid media after 168 hrs. While, minimum 40.00 per cent mortality was observed at 5,000 IJs recovered from wheat flour media.

Experiments with *S. litura* showed that the insects were significantly susceptible to the infection by *S. carpocapsae* population multiplied on animal protein based media (Nutrient broth) than plant protein based media (Wheat flour). Results showed that EPN were more virulent and effective against insect pests. Nematode dosage at higher concentrations yielded highest insect mortality as compared to the lower dosages.

The present findings are similar to the finding of Ali and Ahmad (2009) who have conducted similar experiments in pot and field conditions and concluded that a dose of 200-500 IJs of *Steinernema masoodi* per 100 g of soil was sufficient to bring down the mortality of soil dwelling stage (pupa) of *Helicoverpa armigera* and the adult emergence from pupa. At this dose the adult emergence was only 15-25% as compared to 95% in control. Under field condition, a dose of 6×10^9 IJs of *S. masoodi*

Table 1: Bio-efficacy of *S. carpocapsae* recovered from different artificial media against *S. litura* infecting tomato under pot conditions

Population	Treatments	Mean per cent mortality at different time intervals (hrs)													
		12hrs	24hrs	36hrs	48hrs	60hrs	72hrs	84hrs	96hrs	108hrs	120hrs	132hrs	144hrs	156hrs	168hrs
Nutrient broth	T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	10.00(13.83)	12.50(20.47)	15.00(22.50)	17.50(24.53)	20.00(26.57)	25.00(29.89)	27.50(31.55)	30.00(33.21)	35.00(36.22)	40.00(39.23)	47.50(43.56)
Media	T_2	0.00(0.00)	0.00(0.00)	0.00(0.00)	12.50(20.47)	15.00(22.50)	17.50(24.53)	20.00(26.57)	25.00(29.89)	30.00(33.21)	32.50(34.77)	35.00(36.22)	40.00(39.23)	47.50(43.56)	55.00(47.88)
	T_3	0.00(0.00)	0.00(0.00)	5.00(9.22)	15.00(22.50)	17.50(24.53)	20.00(26.57)	25.00(29.89)	30.00(33.21)	35.00(36.22)	37.50(37.73)	40.00(39.23)	45.00(42.12)	55.00(47.88)	67.50(55.28)
	T_4	0.00(0.00)	0.00(0.00)	7.50(13.83)	17.50(24.53)	20.00(26.57)	22.50(28.23)	30.00(33.21)	35.00(36.22)	40.00(39.23)	42.50(40.67)	47.50(43.56)	55.00(47.88)	65.00(53.78)	80.00(63.43)
Wheatflour media	T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	2.50(4.61)	5.00(9.22)	7.50(13.83)	10.00(18.43)	12.50(20.47)	17.50(24.53)	20.00(26.57)	27.50(31.55)	30.00(33.21)	35.00(36.22)	40.00(39.23)
	T_2	0.00(0.00)	0.00(0.00)	0.00(0.00)	5.00(9.22)	7.50(13.83)	10.00(18.43)	12.50(20.47)	20.00(26.57)	22.50(28.23)	25.00(29.89)	30.00(33.21)	35.00(36.22)	40.00(39.23)	47.50(43.56)
	T_3	0.00(0.00)	0.00(0.00)	2.50(4.61)	7.50(13.83)	10.00(18.43)	12.50(20.47)	15.00(22.50)	25.00(29.89)	27.50(31.55)	30.00(33.21)	35.00(36.22)	40.00(39.23)	45.00(42.12)	55.00(47.88)
	T_4	0.00(0.00)	0.00(0.00)	5.00(9.22)	10.00(18.43)	12.50(20.47)	15.00(22.50)	17.50(24.53)	30.00(33.21)	32.50(34.77)	35.00(36.22)	40.00(39.23)	45.00(42.12)	55.00(47.88)	70.00(56.79)
Maizeflour media	T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	2.50(4.61)	5.00(9.22)	7.50(13.83)	10.00(18.43)	15.00(22.50)	17.50(24.53)	22.50(28.23)	27.50(31.22)	30.00(33.21)	35.00(36.22)	42.50(40.67)
	T_2	0.00(0.00)	0.00(0.00)	0.00(0.00)	5.00(9.22)	7.50(13.83)	10.00(18.43)	15.00(22.50)	20.00(26.57)	25.00(29.89)	27.50(31.22)	30.00(33.21)	35.00(36.22)	40.00(39.23)	50.00(45.00)
	T_3	0.00(0.00)	0.00(0.00)	2.50(4.61)	7.50(13.83)	10.00(18.43)	12.50(20.47)	17.50(24.53)	25.00(29.89)	30.00(33.21)	32.50(34.72)	35.00(36.22)	40.00(39.23)	45.00(42.12)	60.00(50.76)
	T_4	0.00(0.00)	0.00(0.00)	5.00(9.22)	10.00(18.43)	12.50(20.47)	15.00(22.50)	20.00(26.57)	30.00(33.21)	35.00(36.22)	37.50(37.73)	42.50(40.67)	47.50(43.56)	57.50(49.32)	72.50(58.45)
LipidMedia	T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	5.00(9.22)	7.50(13.83)	10.00(18.43)	15.00(22.50)	17.50(24.53)	20.00(26.57)	25.00(29.89)	30.00(33.21)	32.50(34.72)	37.50(37.73)	45.00(42.12)
	T_2	0.00(0.00)	0.00(0.00)	0.00(0.00)	7.50(13.83)	10.00(18.43)	12.50(20.47)	17.50(24.53)	20.00(26.57)	25.00(29.89)	27.50(31.22)	32.50(34.72)	37.50(37.73)	42.50(40.67)	52.50(46.44)
	T_3	0.00(0.00)	0.00(0.00)	2.50(4.61)	10.00(18.43)	12.50(20.47)	15.00(22.50)	20.00(26.57)	25.00(29.89)	32.50(34.72)	35.00(36.22)	37.50(37.73)	42.50(40.67)	47.50(43.56)	65.00(53.78)
	T_4	0.00(0.00)	0.00(0.00)	5.00(9.22)	12.50(20.47)	15.00(22.50)	17.50(24.53)	22.50(28.23)	32.50(34.72)	37.50(37.73)	40.00(39.23)	45.00(42.12)	50.00(45.00)	60.00(50.76)	75.00(60.11)
Modified Wout's media	T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	7.50(13.83)	10.00(18.43)	12.50(20.47)	17.50(24.53)	20.00(26.57)	22.50(28.43)	25.00(29.89)	30.00(33.21)	32.50(34.72)	37.50(37.73)	45.00(42.12)
	T_2	0.00(0.00)	0.00(0.00)	0.00(0.00)	10.00(18.43)	12.50(20.47)	15.00(22.50)	20.00(26.57)	22.50(28.23)	27.50(31.55)	30.00(33.21)	32.50(34.72)	37.50(37.73)	47.50(43.56)	52.50(46.44)
	T_3	0.00(0.00)	0.00(0.00)	5.00(9.22)	12.50(20.47)	15.00(22.50)	17.50(24.53)	22.50(28.23)	27.50(31.55)	32.50(34.72)	37.50(37.73)	40.00(39.23)	47.50(43.56)	52.50(46.44)	65.00(53.78)
	T_4	0.00(0.00)	0.00(0.00)	7.50(13.83)	15.00(22.50)	17.50(24.53)	20.00(26.57)	27.50(31.55)	32.50(34.72)	37.50(37.73)	40.00(39.23)	45.00(42.12)	52.50(46.44)	62.50(52.27)	77.50(61.77)
Control		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEm±		0.00	0.00	1.471	1.433	1.112	0.926	0.674	0.580	0.572	0.564	0.487	0.518	0.508	0.516
CD 5%		0.00	0.00	4.158	4.050	3.144	2.617	1.904	1.639	1.611	1.594	1.375	1.463	1.434	1.458

per plot of 50 X 50 cm when applied to soil, 12% adult emergence was observed against 92% in control thus indicating that EPNs can effectively kill the soil dwelling stage (pupa) of the insect.

Similar, studies in this regards were made by previous workers, Yadav *et al.* (2008) during an experiment on management of *H. armigera* using *S. carpocapsae* found that in foliar application maximum mean per cent mortality (41.67) was recorded at 600 IJs/plant after 5th day of inoculation. However, minimum per cent mortality (2.50) was recorded at 200 IJs as foliar application. Ahmad *et al.* (2010) investigated the susceptibility of 23 insect pests against entomopathogenic nematode, *Steinernema masoodi* under laboratory conditions. The most susceptible insects with 76 to 100% mortality were the greater wax moth (*Galleria mellonella*), rice meal moth (*Corcyra cephalonica*), gram pod borer (*Helicoverpa armigera*), lesser grain borer (*Rhizopertha dominica*), diamondback moth (*Plutella xylostella*), brinjal shoot & fruit borer (*Leucinodes orbonalis*), plume moth (*Exalastis atomosa*) and termite (*Odontotermes obesus*).

In the present study, the mortality of insect larvae increased with an increase in the inoculum levels and period of exposure. Our results are in conformity with Umamaheswari *et al.* (2006) evaluated the efficacy of *Heterorhabditis indica* and *Steinernema glaseri* on *S. litura* under glass house and micro plot conditions on black gram (*Vigna mungo*) and found that mortality increased with increased dosage and exposure time. The maximum mortality was observed with *H. indica* at 5x10^9 IJs/ha after 72 hrs of treatment under glasshouse (75.00) per cent and (50.60) per cent under micro plot conditions. *H. indica* was found more effective than *S. glaseri* in the management of *S. litura*. Yadav *et al.* (2006) conducted an experiment to work out the bio-efficacy of *Steinernema carpocapsae* against *Helicoverpa armigera* infesting gram. Results showed that in foliar application maximum (41.67) per cent mortality was recorded at 600 IJs/plant. Similarly, Kamali *et al.* (2013) conducted laboratory and greenhouse experiments to determine the infectivity of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* against the cucurbit fly, *Dacus ciliates* and found that both the species of EPNs although effective against adult flies but *S. carpocapsae* caused highest adult mortality.

It may be concluded that EPNs are effective biological control agents of numerous soil-dwelling insect pests including coleopteran and lepidopteron. The differences in the rate of nematode infection and insect mortality under various experimental conditions are attributed to the difference in the behavior, virulence, rate of penetration and host searching abilities of nematodes. Rates of nematode infection and insect mortality is dose dependent and varies depending on the type of substrate, insect and experimental conditions.

Statistical analysis

The data collected from the present investigation were statistically analyzed and significance of results was tested. For above experiments, completely randomized design was followed. Means of all experiment were used to compare the efficacy of treatment. ANOVA (Analysis of variance) has been presented in appendices. Some characters have been presented graphically to give a better understanding of

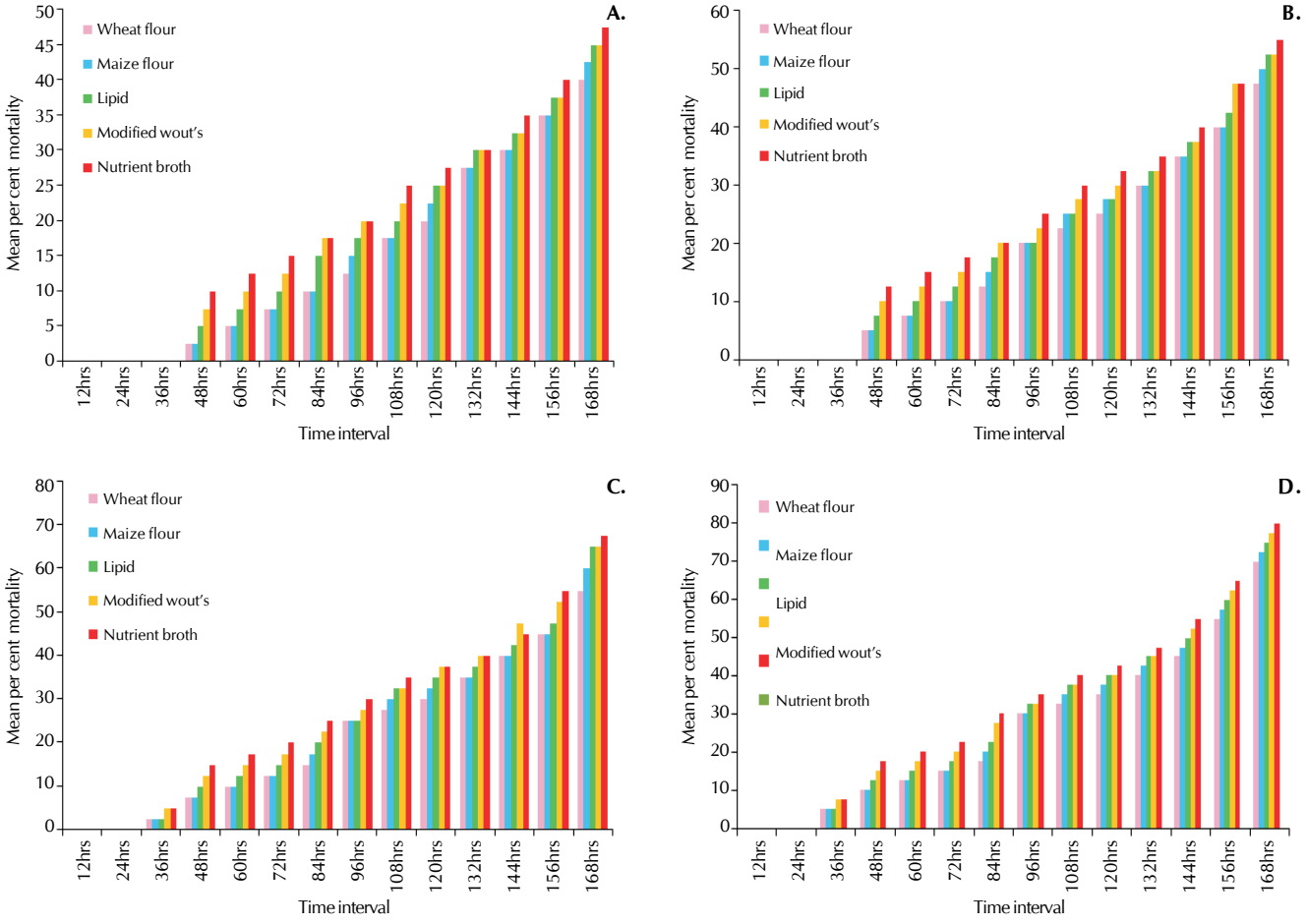

Figure 1: Bio-efficacy of *S. carpocapsae* recovered from different artificial media at different inoculum levels (A. 5000, B. 10000, C. 15000 and D. 20000 IJs/plant) against *S. litura* infecting tomato under pot conditions

important trends wherever necessary. Per cent IJs survival data were analyzed by multifactor ANOVA followed by Duncan's multiple range tests ($P < 0.05$) for separation of means. Figures in parentheses are arc sin transformed values.

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