Responses of Cucurbit Fruitfly, *Zeugodacuscucurbitae*Coquilette to Gel Formulations of Protein Bait

ABSTRACT

In the integrated pest management of fruit flies, trapping is the most practical and effective component. Currently, cucurbit fruit flies are being trapped by employing the male attractant, cuelure. Protein baits, as they attract both male and female fruit flies, they may be explored for the management of fruit flies. In the present study, protein baits were compared with the cuelure for their attraction to fruit flies by electroantennogram experiments. In field conditions, effectiveness of protein bait is restricted due to water loss by evaporation thus making the bait dry. In view of this,studies wereconducted to compare the preferences of fruit flies to protein baits &cuelure and to evaluate the advanced gel formulations of protein bait in attracting fruit flies.In electroantennogram studies, female fruit fly response was found to be high to proteinex bait volatiles followed by soybean bait and cue lure volatiles. Responses of male fruit flies were uniform to proteinex bait and cue lure followed by soybean bait. Among the gel formulations of liquid proteinex bait, in olfactometer, attraction of pectin and sodium alginate gel formulations was equal to the attraction of liquid protein bait. These were followed by xanthan gum powder and carrageenan powder gel formulations. In choice test, pectin powder gel formulation attracted more number of fruit flies and was on par with the attraction of liquid proteinex bait. Luring capacity of carrageenan, sodium alginate and xanthan gum gel formulations were also on par with the liquid proteinex bait.

Keywords: Cucurbit fruit fly – Protein baits – Cuelure – Electro antennogram responses - Gelformulations

1. INTRODUCTION

The cucurbit fruit fly, *Zeugodacuscucurbitae*Coquillett (Diptera: Tephritidae) is the most devastating pest of cucurbitaceous vegetables and fruits in various regions of the world (Kapoor, 1993) and is considered as an important agricultural pest affecting a variety of cultivated fruits and vegetables. It is mainly polyphagous but oligophagous populations have also been found in Thailand, Malaysia and France (Clarke *et al.*, 2001; Vayssières*et al.*, 2008; Hafsi *et al.*, 2016). It attacks 61 species of plants from 19 distinctfamilies with 28 of them are cucurbits and the rest are non-cucurbit hosts (De Meyer *et al.*, 2015). Fruit fly damages the economic part of the crop by oviposition, larval feeding on ovaries&fruit pulp and rottening of damaged fruits (Viraktamath*et al.*, 2003).Inspite of direct losses, indirect losses were also caused in the form of rejection of export produce due to the presence of fruit fly maggots as quarantine restrictions and eradication procedures are very strict (Badii*et al.*, 2015).During our previous research work, protein baits *i.e.*,soybean,casein,proteinex& yeast and food baits *i.e.*,banana, bitter gourd, pineapple, tomato & guava were evaluated in laboratory and field conditions and concluded that proteinexand soybean protein baits were the most attractive to*Z.cucurbitae*. Vargas *et al.*(2002) indicated that the attempts to control

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pestiferous fruit fly populations around the world have depended highly on the use of protein baits combined with a toxicant for several years. Soon after emergence, both male and female fruit flies require proteinaceous diet to survive and reach sexual maturity. Females have a stronger preference for protein sources than males (Drew and Yuval, 1999). So, utilizing protein source bait traps is an effective behavioural based approach which mainly targets female fruit flies since they require protein for successful egg production (Epsky*et al.,* 2014).

Presently cuelure, the male attractant is being used effectively in field conditions to attract and kill the adult fruit flies. However, protein baits can attract both sexes which is an added advantage.But in field conditions, protein bait effectiveness is limited as they are prone to evaporation resulting in dryness of bait which after, fruit flies will not be attracted to the bait. In this background, the present study was undetaken to evaluate the olfactory responses of fruit flies to protein baits in comparison with cuelure and to test the durability of proteinex bait gel formulations in field conditions at Agricultural College and Research Institute, TNAU, Madurai, Tamil Nadu.

2. MATERIALS AND METHODS

2.1. Electroantennogram studies

Electroantennogram experiments on olfactory responses of melon fruit flies to the protein baits (proteinex and soybean baits) were conducted in National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru using electroantennography (EAG) apparatus.In these experiments, response of male and female fruit flies to the eluted volatile samples collected from the proteinex and soybean baits were compared with the standard parapheromone, cuelure.

2.1.1. Collection of volatiles from the protein baits by a volatile collection unit (Dynamic Headspace Sampling Unit)

In the volatile collection unit, the glass jar was connected with an air supply system. An air inlet and outlet were linked to the chamber with the aim of cleaning the chamber using the air from the supply system. A vacuum system was connected to the jar to create vacuum before the collection of volatiles to avoid. A provision was made available in the lower part of the glass chamber for the attachment of volatile trap.

The volatiles from proteinex and soybean bait were collected using the dynamic head space sampling unit. Before the initiation of the process, the glass jar was cleaned by rubbing with the cotton soaked with HPLC grade dichloromethane. Then air was pumped into the glass chamber for about 45 minutes to remove any odours present in the chamber and then vacuum was created inside for 30 minutes. The days at which the proteinex and soybean baits

attracted more number of fruit flies in olfactometer studies were selected to get the maximum amount of volatiles. Air was pumped into the jar through activated charcoal for 45 minutes. The bait volatiles were trapped in a volatile trap containing 50 mg of Porapak Q chemical absorbent (adsorption tube) that was attached to glass jar separately. Volatiles were collected for 3 hours.The volatiles adsorbed in the trap (Porapak Q) were extracted using 10 ml HPLC grade dichloromethane and stored at -20^{o}C.

2.1.2. Electroantennogram experiments

Electroantennogram (EAG) responses of male and female *Z.Cucurbitae* were recorded using a commercially available electroantennographic system (Syntech, Hilversum, The Netherlands) available at National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. The EAG consisting of a dual electrode probe for antenna fixation, a CS-05 stimulus controller and an IDAC 232 box for data. The antenna was fixed with the tip of one of the electrodes and scape was fixed to the other electrode as suggested by Reinecke *et al.,* (2005). The antenna was fixed between the two electrodes using Spectra 360 conductive gel (Parker, Orange, New Jersey). The antenna was flushed continuously with stream of activated charcoal filtered air. Response of *Z. cucurbitae* male and female fruit fly (10 nos. each) antennae to the volatile components collected from proteinexand soybean baits was recorded using an electroantennographic system (Syntech). Electroantennogram responses of fruit flies to the volatiles of protein baits were compared with thecuelure and dichloromethane (solvent used for eluting the protein bait volatiles)

2.2. Evaluation of the attraction of proteinex bait gel formulations to melon flies

2.2.1. Olfactometer studies

Olfactometer studies were conducted to evaluate the attraction of gel formulations of proteinex bait to melon flies in comparison with the liquid proteinex bait.

Table 1. Treatments details

S. No.	Particulars	Ingredients
1	Liquid proteinex bait	Proteinex powder (10%) + jaggery (10%) + ammonium acetate (5%) + borax (2%)
2	Xanthan gum powder gel	Xanthan gum powder (0.6%) + liquid

	formulation	protinex bait
3	Carrageenan powder gel formulation	Carrageenan powder (3.6%) + liquid protinex bait
4	Guar gum powder gel formulation	Guar gum powder (1.6%) + liquid protinex bait
5	Pectin powder gel formulation	Pectin powder (0.8%) + liquid protinex bait
6	Sodium alginate gel formulation	Sodium alginate (1.8%) + liquid protinex bait
7	Gum Arabic powder gel formulation	Gum arabic powder (7.3%) + liquid protinex bait
8	Untreated control	Water

Sponges saturated with liquid proteinex bait, gel bait and water (untreated control) were kept in three odour containers of the olfactometer and the fourth arm was blocked with cotton and no air supply was provided. Activated charcoal-filtered air was pumped inside the olfactometer arms for 45 minutesto remove any odours present inside. Later, through the suction pump, vacuum was created inside the olfactometer for 30 minutes. Then the sponges saturated with the samples were kept in the odour containers and again filtered pure air was passed for 45 minutes. Afterwards, 12 hours prestarvedfruit flies (30 nos.) were released into the olfactometer through the upper detachable lid.

During the conduct of experiment, olfactometer was covered with a a dark red colour cloth to avoid the phototrophic effect on the attraction of fruit flies. Each treatment was replicated thrice and during each replication, direction of olfactometer was changed. Experiments were conducted with each treatment separately and replicated thrice. Number of adult flies attracted to the odour zones of respective samples was recorded uptoone hour at 10 minutes interval and the observation at one hour was considered as the final.

2.2.2. Gated cup trap studies

A choice test was conducted using gated cup traps. Gated cup traps were prepared by covering the 100 ml glass beaker containing 50 ml of gel formulation with the aluminium foil in which a central cut was made. The lower end and the plastic lid of an Eppendorf tube (0.7 cm diameter) were removed and inserted in the central cut in the aluminium foil which restricted the adult flies from escaping once they enter the trap.

All the gated cup traps with different gel formulations of proteinex bait were placed in the insect cage (25x25x25 cm) with equal distance in between. 50 fruit flies were released in the centre of the cage. Number of fruit flies trapped inside the gated cup traps was recorded after 24 hours of release of the insects. This experiment was replicated thrice.

2.3. Stability test

All the proteinex powder gel formulations were poured in the fruit fly traps @ 200 ml /trap, tied in the partial shade conditions to mimic the natural habitat of cucurbit field ecosystem. Reduction in the weight of each bait due to evaporation was recorded daily up to 15 days in all the baits.

2.4. Statistical Analysis

Data were subjected to appropriate transformations before analysis. Means were separated by Tukey's HSD test. Statistical analyses were performed using SPSS Windows (version 22.0) (IBM Corp. Released 2013) (Gomez and Gomez, 1984).

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3. RESULTS AND DISCUSSION

3.1. Evaluation of olfactory responses of melon fruit flies to protein baits - electroantennogram (EAG) experiments

Electroantennogram responses of female fruit flies to the volatiles of protein baits revealed that they responded more to the volatiles of proteinex bait (0.3729 mV) (Table 2)followed by soybean bait (0.1389 mV) and cue lure (0.1334 mV) and both these responses were statistically on par. Olfactory responses of male fruit flies revealed their equal response to proteinex bait (0.2667 mV) and cue lure (0.2434) (Table 2)followed by soybean bait (0.1784 mV).Female fruit fly response was high to the volatiles of proteinex bait followed by soybean bait and cue lure. Olfactory responses of male fruit flies revealed uniform response to proteinex bait and cue lure followed by soybean bait. Positive electroantennogram responses of female fruit fly to proteinex bait volatiles confirmed it's luring potency to fruit flies. As response of male fruit flies to proteinex bait was on par with the responses of cue-lure, this aspect may be further studied for utilizing the proteinex bait volatiles in trapping fruit flies at field level.

Table 2. Electroantennogram studies on the attraction of melon fruit flies to protein baits

Treatments	EAG responses (amplitude) of antennae of female fruit flies (mV)										
	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean*
Dichloro methane	0.139	0.11	0.003	0.05	0.003	0.000	0.072	0.000	0.029	0.007	0.0413 (0.2032)[b]
Cue lure	0.257	0.228	0.136	0.15	0.084	0.045	0.085	0.118	0.066	0.165	0.1334 (0.3652)[ab]
Protinex bait	1.24	1.211	0.309	0.199	0.145	0.046	0.191	0.049	0.215	0.124	0.3729 (0.6107)[a]
Soybean bait	0.32	0.291	0.243	0.158	0.044	0.052	0.112	0.047	0.076	0.046	0.1389 (0.3727)[ab]
										CD (0.05)	0.29
										S.E (d)	0.09
Treatments	**EAG responses (amplitude) of antennae of male fruit flies (mV)**										
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**Mean***
Dichloro methane	0.047	0.038	0.017	0.048	0.001	0.029	0.014	0.04	0.001	0.026	0.0261 (0.1616)[b]
Cue lure	0.164	0.648	0.327	0.211	0.065	0.403	0.191	0.179	0.046	0.2	0.2434 (0.4934)[a]
Protinex bait	0.107	1.06	0.464	0.141	0.039	0.344	0.075	0.218	0.023	0.196	0.2667 (0.5164)[a]
Soybean bait	0.158	0.441	0.219	0.321	0.101	0.153	0.107	0.131	0.038	0.115	0.1784 (0.4077)[ab]
										CD (0.05)	0.32
										S.E (d)	0.08

*Mean of ten replications

Figures in parentheses are square root transformed values

Means followed by the same letter in a column are not significantly different by Tukey's HSD test. (P=0.05)

3.2. Attraction of proteinexgel formulations to melon fruit flies

3.2.1. Olfactometer studies

Among the gel formulations of liquid protinex bait, attraction to melon flies was high in pectin gel formulation followed by sodium alginate gel formulation with 9.00 and 8.67 fruit flies/odour arm respectively. These two treatments were on par with the attraction of liquid proteinex bait (9.00 fruit flies/odour arm). Next were, xanthan gum powder and carrageenan powder gel formulations with 7.67 and 7.00 fruit flies/odour arm respectively. Comparatively, least fruit fly attractant formulations were guar gum powder and gum arabic powder gel formulations with 6.67 fruit flies/odour arm (Table 3).

Table 3. Olfactory responses of fruit flies to different gel formulations of proteinex bait

S. No	Treatments	Number of fruit flies attracted/odour arm*					After one hour
		10 min.	20 min.	30 min.	40 min.	50 min.	
1	Xanthan gum powder gel formulation	5.00	6.33	5.67	7.67	5.67	7.67 (2.76)^{ab}
2	Carrageenan powder gel formulation	2.67	2.67	4.67	5.67	6.67	7.00 (2.65)^{ab}
3	Guar gum powder gel formulation	4.00	4.00	5.67	6.00	7.00	6.67 (2.58)^{b}
4	Sodium alginate gel formulation	5.33	5.00	5.00	6.67	7.00	8.67 (2.94)^{a}
5	Pectin powder gel formulation	4.33	5.00	6.33	6.67	8.33	9.00 (3.00)^{a}
6	Gum arabic powder gel formulation	3.33	2.67	3.67	4.67	6.00	6.67 (2.58)^{b}
7	Liquid protinex bait formulation	3.67	5.67	6.00	6.00	9.00	9.00 (3.00)^{a}
8	Untreated control	1.67	1.78	2.44	1.56	2.00	1.39 (1.18)^{c}
						CD (0.05)	0.40
						S.E (d)	0.10

*Mean of three replications

Figures in parentheses are square root transformed values

Means followed by same letter in a column are not significantly different by Tukey's HSD test. (P=0.05)

3.2.2. Gated cup trap studies in choice conditions

Among the gel formulations of liquid protinex bait, after 24 hours, more number of fruit flies (8.33) (Table 4) were recorded in pectin gel formulation gated cup traps and this count was more than the liquid proteinex bait (6.67 fruit flies). Luring capacity of carrageenan powder, sodium alginate and xanthan

gum powder formulations were found to be on par with the liquid proteinex bait with 6.00, 4.33 and 4.00 fruit flies in their gated cup traps respectively. Guar gum and gum arabicpowder formulations attracted comparatively less number of fruit flies *i.e.*, 3.33 and 3.00 respectively.

Table 4. Attraction of fruit flies to gel formulations of proteinex bait – Gated cup trap studies – Choice conditions

S. No.	Treatments	Number of fruit flies trapped in gated cup trap after 24 hours			
		I set	II set	III set	Mean*
1	Proteinex liquid bait	6.00	6.00	8.00	6.67 (2.58)^{ab}
2	Xanthan gum powder gel formulation	3.00	4.00	5.00	4.00 (2.00)^{ab}
3	Carrageenan powder gel formulation	4.00	6.00	8.00	6.00 (2.43)^{ab}
4	Pectin powder gel formulation	8.00	8.00	9.00	8.33 (2.89)^{a}
5	Sodium alginate gel formulation	5.00	6.00	2.00	4.33 (2.03)^{ab}
6	Guar gum powder gel formulation	3.00	2.00	5.00	3.33 (1.79)^{b}
7	Gum arabic powder gel formulation	3.00	2.00	4.00	3.00 (1.72)^{b}
8	Untreated	0.00	0.00	1.00	0.33 (0.33)^{c}
				CD (0.05)	1.15
				S.E(d)	0.31

*Mean of three replications
Figures in parentheses are square root transformed values
Means followed by the same letter in a column are not significantly different by Tukey's HSD test. (P=0.05)

3.3. Evaluation of stability of gel formulations of liquid proteinex bait

At 5 days after placement of traps (DAP), weight reduction was more (49.77%) in liquid proteinex baitand lost approximately 50 per cent of it's water content. Weight loss in proteinex bait gel formulations ranged from 10.25% (guar gum powder gel formulation) to 17.54% (gum arabic powder gel formulation). Among the proteinex bait gel formulations, weight loss was less (10.25%) in guar gum powder and pectin powder gel formulations (10.87%) (Table 5)and both were on par with each other. Succeeding these, weight loss was less in carrageenan powder (11.25%) and xanthan gum powder gel formulations (13.92%). Among the gel formulations, evaporation and weight loss was less in guar gum powder and pectin powder gel formulations.

At 10 DAP, 65.61 per cent weight loss was observed in proteinex liquid bait. Less per cent reduction in weight loss was recorded in pectin powder (25.10%) and guar gum powder gel formulations (25.64%) and both were statistically on par. Weights of the proteinex liquid bait and gum arabic powder gel formulation were reduced considerably *i.e.,* 65.61 and 46.49 per cent respectively. At 15 DAP, weight of the proteinex liquid bait reduced markedly (77.82%). Among the gel formulations, weight reduction ranged between 49.37% (pectin powder gel formulation) and 66.66% (gum arabic powder gel formulation).

Comparative to liquid proteinex bait, in gel formulations, evaporation was less and quantity of bait was not reduced considerably in traps. Among the gel formulations of liquid protinex bait, pectin powder and guar gum powder gel formulations were less evaporative up to ten days after placement of traps. These were closely followed by carrageenan powder and xanthan gum powder gel formulations. Less evaporation observed in gel formulations is a positive point in maintaining these bait traps in the field conditions up to 10 days. Inspite of good attraction in liquid protinex bait traps, high evaporation in field made it less attractive to fruit flies. In this context, converting liquid proteinex bait to pectin or guar gum powder gel formulations increases it's efficiency in trapping more number of fruit flies. These formulations can be maintained in the field upto 10 days without adding water. After that also by adding water, it can be maintained another five days. Among the gel formulations, attraction to fruit flies was more in pectin powder, sodium alginate, xanthan gum powder and carrageenan powder gel formulations. Among the above three, in pectin powder and guar gum powder gel formulations, evaporation was comparatively less up to 10 DAP. Hence, these formulations of proteinex bait can be placed in field for luring cucurbit fruit flies effectively.

Gelling agents are essential components in insect diets because they keep water in a solid state, avoid interactions between ingredients, and preserve the blended condition of the ingredients. Some gel forming agents (proteins, starches and pectin) can be used, whereas others (agar and carrageenens) are indigestible. Carbohydrates are the most frequently used gelling agents in food which includes gums such as guar gum and carboxymethylcellulose, carrageenan, agar, starch, alginates and pectins (Cohen, 2003). Guar gum is extracted from the seeds of cluster bean, *Cyamopsis tetragonolobus* and contains guaran (85%), a nontoxic colloidal polysaccharide with a water-soluble property (Imeson, 1997; Jain *et al.*, 2005).

Table 5. Stability test of gel formulations of proteinex bait

S. No.	Treatments	Weight (g)															Reduction in weight (%) from I to XV DAP		
		I DAP	II DAP	III DAP	IV DAP	V DAP	VI DAP	VII DAP	VIII DAP	IX DAP	X DAP	XI DAP	XII DAP	XIII DAP	XIV DAP	XV DAP	V DAP	X DAP	XV DAP
1	Protinex liquid bait	221	201	173	157	120	111	102	91	87	79	68	61	58	53	49	49.77 (44.87)^{a}	65.61 (54.10)^{a}	77.82 (61.92)^{a}
2	Xanthan gum powder gel formulation	237	225	218	210	204	198	189	181	175	169	129	121	118	113	109	13.92 (21.91)^{c}	28.69 (32.39)^{d}	54.00 (47.30)^{d}
3	Carrageenan powder gel formulation	231	228	221	217	205	201	195	188	181	170	135	127	120	115	111	11.25 (19.60)^{d}	26.40 (30.92)^{de}	51.94 (46.11)^{de}
4	Pectin powder gel formulation	239	234	227	220	213	207	199	191	185	179	167	152	147	133	121	10.87 (19.25)^{d}	25.10 (30.07)^{e}	49.37 (44.64)^{e}
5	Sodium alginate gel formulation	219	211	207	195	183	171	169	162	158	142	131	127	112	107	101	16.43 (23.91)^{b}	35.15 (36.36)^{c}	53.88 (47.23)^{c}
6	Guar gum powder gel formulation	234	224	219	215	210	201	198	191	187	174	154	147	138	127	116	10.25 (18.67)^{d}	25.64 (30.42)^{e}	50.42 (45.24)^{e}
7	Gum arabic powder gel formulation	228	213	205	191	188	171	158	145	138	122	110	103	97	89	76	17.54 (24.76)^{b}	46.49 (42.99)^{b}	66.66 (54.73)^{b}
																CD (0.05)	20.21	10.98	7.19

*Mean of three replications

Figures in parentheses are arc sine root transformed values

Means followed by the same letter in a column are not significantly different by Tukey's HSD test. (P=0.05)

4. CONCLUSION

Electroantennogram studies on the olfactory responses of cucurbit fruit flies to protein bait volatiles in comparison with cuelurerevealed that, female fruit flies respondedmore to proteinex bait volatiles followed by soybean bait and cue lure volatiles. Male fruit flies responded uniformly to proteinex bait and cue lure followed by soybean bait. Olfactometer studies on the attraction of gel formulations of liquid proteinex bait revealed that, attraction of pectin and sodium alginate gel formulations was equal to that of liquid proteinex bait. Next to these were xanthan gum powder and carrageenan powder gel formulations. In gated cup trap choice tests also, pectin powder gel formulation was found to be the most attractive to fruit flies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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