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Response of *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae) to *Brassica* extracts

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Abstract
Plant volatiles are known to play very important in host finding for insect pests. These bioactive volatiles evoke EAGs responses in insect antennae. The antennal sensitivity of *Pieris brassicae* was recorded against volatiles of *Brassica* species by electroantennogram technique. A dose-dependent response was found to each extracts or and maximum EAG response was elicited by 1% concentration. The adult had a strong sensitivity to *Brassica carinata* (0.88 mV) and *B. juncea* (0.79 mV) than other species. However, no significant difference was found among the response evoked by *Brassica* species in the antennae of butter fly (Tukey's test, $p<0.01$). The results of present study suggest the antennal sensitivity of *P. brassicae* to a range of concentrations of each species. Further research is needed to investigate effects of these compounds on natural enemies of *P. brassicae*.

Keywords: *Brassica*, EAG, *Pieris brassicae*, volatiles, host-plant interactions

Introduction
Pieris brassicae (Lepidoptera: Pieridae) is commonly called as large the cabbage white, cabbage butter fly or cabbage moth. It is widely distributed pest of cruciferous crops [1]. The newly hatched larvae feed on leaves by scrapping chlorophyll and in later stage total defoliation of the leaf was noticed. Up to 92 per cent reduction in crop yield by *P. brassicae* has been reported [2]. The management of this was mostly obtained by application of chemical insecticides. But frequent application of insecticides has led the development of resistance, secondary pest outbreak and also affects the associated communities [3]. The identification and quantification of these volatiles have grown the interest of biologist and chemical ecologist as these compounds serve not only as aromatherapeutic agents but also play significant role in arthropod plant interaction.

Plant odours present a true picture of the plant health i.e., indicating whether it is under any kind of physiological stress. The plants are known to emit some or other volatile compounds in response to feeding damage caused by insects [4]. They are classified into different group based on biological functions [5]. A large number of compounds including glucosinolates are produced by *Brassica* plants [6, 7]. Glucosinolates are the major metabolites of *Brassica* that affect behavior of associated insects [8]. These volatile compounds are used by herbivore insects in process of host plant location [9]. The volatiles of host plant can be identified by electroantennography. In this study, we investigated the effect of *Brassica* extracts on electrophysiological response of *P. brassicae*.

2.0 Materials and methods
2.1 Host plant
Five *Brassica* species (*B. napus, B. nigra, B. rapa, B. juncea* and *B. carinata*) were sown in research farm of IARI (Indian Agricultural Research Institute), New Delhi during 2016. The experiment was laid out following randomized block design with four replications. The whole field was divided into 4×5 m plots. All the agronomic practices were followed as per the recommended package of practices for growing *Brassica*.

2.2 Insect
The newly hatched larvae of *Pieris brassicae* with leaves were collected from *Brassica* field and transferred into the jar (20×10 cm). The jar with field collected larvae were cover with a muslin cloth and tightly secured by rubber band. The temperature of the rearing room was maintained at 27 ± 1 ºC with 65 ± 5% RH.

The culture of *P. brassicae* was checked daily and fresh leaves were provided from field to maintain the food requirement. The pupae of *P. brassicae* can be seen on the sidewalls of the jar. The newly emerged adults were collected from the jar and used for further studies.

2.3 Preparation of plant extracts

The host plant extract of each species were prepared based on the protocol used by Kumar *et al.* [10, 11] The sample of each species was taken into the 250 mL of conical flask and 150 mL of chilled n-hexane was poured into sample flask. Conical flask with sample was kept in refrigerator for 24 hrs. Then it was passed through Whatman no. 1 filter paper to remove the plant materials from the solvent. The obtained elute was further cleanup by passing through glass columns. Finally elute was concentrated at 25-30 ºC with 40-45 RPM by vacuum evaporator and stored at -20 ºC for further studies. This final volume is considered as a stock (100%) solution from which different concentration was prepared.

2.4 Electroantennography

The response of *P. brassicae* adult to the extract of different species was investigated by electroantennography. The EAG setup and protocol used in this experiment was similar to those used by Kumar *et al.* [12] for red cotton bug (*Dysdercus singulatus*). The antennae of butterfly were removed from the base of head by using a sharp knife. Few segments from tip and also from base were clipped off before mounting the antenna between electrodes. Ten micro-litters dose of extracts was used throughout experiment. The data obtained with plant extracts was analyzed by EAG 2000 software (Syntech, Germany) and presented in results.

2.5 Statistical analysis

The obtained value of EAG amplitude (-mv) from antennal receptor of the test insects for each stimuli was subjected to one way ANOVA (Analysis of variance) and the treatment means were separated by Tukey's test (p<0.01).

3. Results and discussion

The EAG response of *P. brassicae* was found in a dose-dependent manner and maximum response was obtained with 1% concentration of each Extract. The antennae of adult were least sensitive to extracts of each species at 0.0001% concentration (Fig. 1). A significant difference was noticed in EAG response between 0.1 and 1% dose. The adult had a strong response to the extracts of *B. carinata*, followed by *B. juncea* and *B. rapa*. However, no significant difference was found among the EAG response of *Brassica* species (Fig. 1).

Selection of plant varieties can directly affect the behaviour of associated herbivores. The composition of volatiles in plants may vary from species to species and also within same plant. In turn, these differences can affect the outcomes of insect-plant interactions [13]. The higher response of butter fly to 1% dose may be due the high concentration and the ability of this concentration to affect the sensilla of antennae. Such dose-dependent response in insect has been reported previously [11, 12]. The present study revealed the antennal sensitivity of *P. brassicae* to host plant extracts. Maximum EAG response in adult was obtained with *Brassica carinata* and *B. juncea.* It is possible that these two species may contain more constitutes than others and these constitutes have great significance in host plant selection process of butter fly, *P. brassicae*. Previous studies showed the importance of plant volatiles in process of host plant interaction [14, 15]. However, the response of insect to plant volatiles depend on the structural and functions adaptation of the olfactory system.

The selection of appropriate plant genotypes may effects the behaviour of associated herbivores and thus could increase the efficiency of bioagents. The results of present study suggest the antennal sensitivity of *P. brassicae* to a range of concentrations of each species. The role of these EAG-active compounds must be investigated in behavioural bioassay to support our findings. Further research is needed to investigate the effects of these compounds on natural enemies of *P. brassicae*.

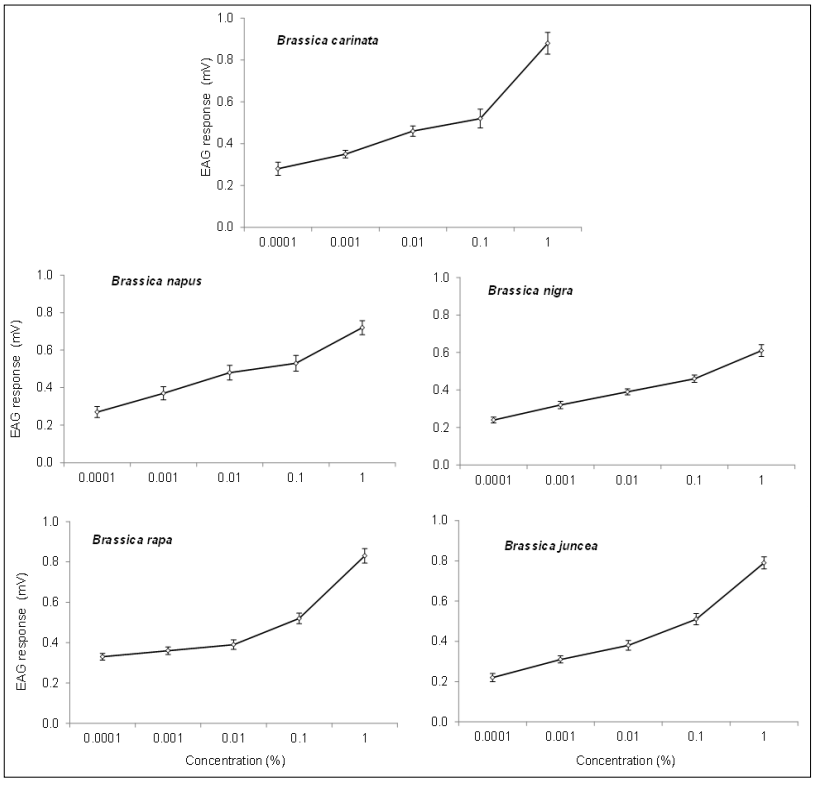

Fig 1: EAG response profile of *P. brassicae* to extracts of *Brassica*. Bars with common letter are not significantly differ (Tukey's test, p<0.01).

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